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QUANTITATIVE CHARACTERISTICS OF THE GYNOECIUM IN *PRIMULA VULGARIS* HUDS

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Abstract

The examination of the quantitative characteristics of the gynoecium in a typical heterostylous species, *Primula vulgaris* points to several differences between the two morphs beyond the well-known ones that are closely related to the phenomenon of heterostyly. Stigma length, stigma width and ovule length differ significantly in the two morphs at $P \leq 0.001$, whereas ovary length, ovary width and ovule number per ovary do not differ in the two morphs. Standard deviation, range and frequency distribution diagrams of most examined traits prove that the pin morph is much more variable than the thrum one. Correlation studies show that the length of the gynoecium is determined only by the style length, and the sizes of the other parts of gynoecium are more or less independent of each other.

Key words: heterostyly, gynoecium, *Primula*

Introduction

Heterostyly is a special form of genetic polymorphisms. Heterostylous plant populations consist of two (distyly) or three (tristyly) morphs with reciprocal arrangement of stigmas and anthers (reciprocal herkogamy). *Primula* species are well-known and widely investigated examples of distyly. The two morphs are easily distinguishable: the pin form has long style and its anthers locate near the middle of the corolla tube, while the thrum form has short style and its anthers are near the top of the corolla tube.

The morphological manifestation of reciprocal herkogamy is connected to several ancillary polymorphisms of both gynoecium and androecium. The gynoe-

cium is built up from three parts: stigma, style and ovary, but most investigations of polymorphisms concern only the style and the stigma. Numerous general and particular instances can be found indicating quantitative and qualitative differences of these two parts of gynoecium.

In *Waltheria viscosissima* (Sterculiaceae) the style of the two morphs varies not only by length but also by shape. The long style is repeatedly curved and bears large verrucae and long hairs, whereas the short style is only slightly curved, has smaller verrucae and bears shorter hairs (KÖHLER, 1973, 1976). In *Primula obconica* (Primulaceae) the histological structure of the style differs in the two morphs: the transmitting tissue of the short style is much larger than that of the long one (DOWRICK, 1956). Difference in the size of the stigma is also a frequent feature in heterostylous species. The receptive surface of the pin morph is typically larger than that of the thrum one, this feature is conspicuous in *Plumbago capensis* (Plumbaginaceae) and *Linum grandiflorum* (Linaceae, DULBERGER, 1992). However there are species where an opposite ratio of stigma size occurs: in *Primula malacoides* (Primulaceae, PANDEY and TROUGHTON, 1974) and *Hedyotis caerulea* (Rubiaceae, ORNDUFF, 1980) the thrum stigma is larger than the pin one. The polymorphism of gynoecium can also be manifested by the shape of the stigmas. In *Rudgea jasminoides* (Rubiaceae) the thrum stigmas are long and narrow, while the pin ones are short and broad (BAKER, 1956). Regarding some *Primula* species (Primulaceae) small differences in the shape of stigmas are also mentioned, for example in *P. vulgaris* (HESLOP-HARRISON *et al.*, 1981), *P. elatior* (SCHOU, 1983) and *P. obconica* (BIR BAHADUR *et al.*, 1984).

In a previous study we examined the morphological differences of some perianth traits in the two morphs of *Primula vulgaris* and *P. veris*. The corolla-tube length proved to be applicable to distinguish the morphs by both means and frequency-distribution diagrams (KÁLMÁN and MIHALIK, 1996). Besides the features of the perianth we have also examined the gynoecium of *Primula* species and quantitative characteristics of stigma, style, ovary and ovules of *Primula vulgaris* are presented in this study.

Materials and methods

The object of our examination is a typical distylous species, *Primula vulgaris* HUDS. The examinations were carried out in a semi-natural population in the Botanical Garden of József Attila University. The plants were planted several years ago and now grow together with numerous herbaceous species constituting a steady community. We have investigated this semi-natural population since 1995 and established that the natural survival of the species is provided by both vegetative and generative reproduction.

For the examinations the flowers were collected from older plants with at least 15 leaves. All the flowers were from different plants and were collected 2–3 days after opening. Gynoeciums were dissected from the flowers and immediately placed onto a wet filter-paper. The gynoeciums were divided

into three parts: stigma, style and ovary and the ovules were removed from the ovaries. By using a binocular stereo-microscope connected to a video camera we took pictures about the stigmas, ovaries and ovules and the pictures were processed by an image-analysing software (Image Pro-Plus 3.0).

The length of the whole gynoecium, the stigma and the ovary were measured along the longitudinal axis of the gynoecium and the width of the stigma and the ovary were measured perpendicularly to the length. The length of the style was calculated by subtracting the sum of stigma and ovary length from the whole length of the gynoecium. The number and the largest length of ovules was also determined. 100–120 gynoeciums and about 500 ovules removed from 10 ovaries were measured in each morph.

In the first step of data processing the frequency-distribution diagrams of the measured characters were created and data which did not fit into the normal distribution were left out. It occurred only in the case of pin stigma length where there were three extremely large values. By using modified data the mean, standard deviation and range (the difference between the largest and smallest values) of each character were calculated. Means of each character in the two morphs were compared by Student's t-test. We calculated the ratio of data if it seemed to be informative and compared the behaviour of the two morphs. Correlations were also checked among the measured features and the equations of the regression lines were calculated.

Results

Characterisation of style, stigma and ovary

The mean, standard deviation and range of measured features in thrum and pin morphs are given in Table 1. and Table 2., respectively. The most striking manifestation of reciprocal herkogamy is the difference in the length of style between the two morphs. The pin style is about twice as long as the thrum one (pin:thrum ratio is 2.17:1). There is no overlap between the two morphs, the longest thrum style is 7. 88 mm, while the shortest pin one is 10. 33 mm. The standard deviation of the pin style length is about twice as big as that of the thrum one and the range of the pin data is half as big again as that of the thrum one. The frequency distribution diagrams are typical of the morphs: in the diagram of thrum data there is a characteristic peak with 50% of all the cases in the category of 6–7 mm, while the diagram of pin data is much flatter, in the middle of the range there are four categories (from 12 mm to 16 mm) with 15–30% of all the cases.

Apart from the difference of style length the difference in stigma size is also mentioned in some heterostylous species. According to our results in *Primula vulgaris* both the length and the width of stigma are different in the two morphs: the pin stigma is longer and wider than that of the thrum one. Comparison of the means by Student's t-test shows that the difference is significant ($P \leq 0.001$). The standard deviations and the ranges are slightly larger in the pin morph than in the thrum one. The frequency distribution diagrams of the stigma length show unambiguous differences between the two morphs. The peaks of the thrum and the pin data are separated sharply: the thrum peak appears in the category of 0.9–1.0 mm, while the pin one is in the category of 1.2–1.3 mm. The frequencies at the

peaks are about 30% of all the cases in both morphs. The separation of thrum and pin peaks is not so clear in the frequency distribution diagrams of stigma width. There is a common peak in the category of 1.3–1.4 mm, but the frequency of pin data in the peak is higher than that of thrum ones (37% of all the cases for pin and 29% of all the cases for thrum morph). In the categories smaller than 1.3 the frequency of thrum data is higher than that of pin ones, while in the categories bigger than 1.4 the arrangement of frequencies is opposite.

Table 1. Mean, standard deviation and range of measured features in thrum morph (St. dev. = standard deviation, N= number of cases)

	length of style (mm)	length of stigma (mm)	width of stigma (mm)	length of ovary (mm)	width of ovary (mm)
Mean	5.87	0.92	1.20	1.95	1.95
St. dev.	0.72	0.13	0.14	0.27	0.19
Range	4.18	0.59	0.79	1.29	0.97
N	117	118	118	121	121

Table 2. Mean, standard deviation and range of measured features in pin morph (St. dev. = standard deviation, N= number of cases)

	length of style (mm)	length of stigma (mm)	width of stigma (mm)	length of ovary (mm)	width of ovary (mm)
Mean	12.76	1.19	1.33	1.90	2.02
St. dev.	1.34	0.16	0.16	0.26	0.22
Range	5.98	0.86	0.83	1.11	1.21
N	102	99	102	120	119

The third part of the gynoecium is the ovary. In *Primula vulgaris* the length and the width of ovary are similar in both morphs, so according to the means the ovary seems to be spherical. Comparison of the means shows that these traits are very similar to each other in the two morphs: there is no significant difference for the ovary length and the difference is very small for the ovary width ($P \leq 0.05$). The standard deviations, the ranges and the frequency distribution diagrams are also almost the same in the two morphs. The only one difference which can be found is that in the middle of the ranges (between 1.8 and 2.4 mm) the frequencies of thrum data are higher than the frequencies of pin ones, whereas at the edges of the ranges the frequencies of the pin data are higher than that of the thrum ones.

Ratios of the measured features

Some ratios seemed to be informative in the exact description of the characteristics of gynoecium. We examined the length:width ratios of stigma and ovary and the length ratios of each part of the gynoecium. The mean, standard deviation and range of the calculated ratios are summarised in Table 3.

The length:width ratio of the stigma is larger in the pin morph than in the thrum one so the thrum stigmas seem to be shorter and/or wider than the pin ones. With respect to the standard deviation and range there is no difference between the two morphs at all, but in the frequency distribution diagrams the data of the two morphs are separated sharply. The peak of the thrum data is in the categories of 0.7–0.9 mm with 60% of all the cases (the pin frequency is 23% of all the cases there), the peak of the pin data is in the categories of 0.9–1.1 mm with 55% of all the cases (the thrum frequency is 30% of all the cases there). The most important difference between the two morphs can be seen when the distributions of frequencies are compared to the 1.0 value, where the length and width are equal, so the stigma is almost spherical. 82% of all the thrum proportions is smaller than 1.0, while 50% of all the pin proportions is equal with or bigger than 1.0.

Table 3. Mean, standard deviation and range of the calculated ratios
(St. dev. = standard deviation, N= number of cases)

	stigma length: stigma width		ovary length: ovary width		stigma length: ovary length		style length: stigma length		style length: ovary length	
	thrum	pin	thrum	pin	thrum	pin	thrum	pin	thrum	pin
Mean	0.77	0.90	1.00	0.94	0.48	0.64	6.53	10.81	3.07	6.89
St. dev.	0.12	0.12	0.11	0.10	0.09	0.12	1.28	2.01	0.59	1.23
Range	0.62	0.62	0.54	0.64	0.48	0.61	7.57	9.71	3.18	5.90
N	118	97	118	120	117	99	117	102	117	102

The length:width ratio of the ovary is obtained opposite to that of the stigma: the thrum ratio is larger than the pin one. Standard deviations and ranges are similar in the two morphs and there is no significant difference between the frequency distribution diagrams either. The peaks are in the category of 1.0–1.1 mm in both morphs, so most of the ovaries are really spherical. In the categories under 1.0 the pin frequencies are higher, while in the categories above 1.0 the thrum ones are higher.

The length ratios of the parts of the gynoecium, namely stigma length:ovary length, style length:stigma length and style length:ovary length ratios were also

calculated. Strikingly in all the three examined ratios the standard deviations and the ranges of pin data are larger than those of thrum ones and the data of the two morphs are separated sharply in the frequency distribution diagrams. In the diagram of stigma length:ovary length ratio the peak of the thrum data is in the category of 0.5–0.6 mm, while the pin peak appears in the category of 0.7–0.8 mm with 40% of all the cases in both morphs. The diagrams of style length:stigma length and style length:ovary length ratios show clearly the general difference between the two morphs: in the diagrams of thrum data there is a definite peak with a high frequency, while the diagrams of the pin data are much flatter, there are more categories with similar frequencies in them.

Correlation and regression

The correlation coefficients of the compared traits are given in Table 4., the most important ones are enhanced in bold. In the first step the correlation between the length of the whole gynoecium and the length of each part of the gynoecium were examined. According to our results there is a strong connection between the whole gynoecium length and the style length, in the diagram representing the style length against the gynoecium length the points fit tightly to the regression lines (Fig. 1.). The slope of the regression line (r) is 0.86 for the thrum data and 0.96 for the pin ones. In contrast to the style length there is no correlation in the case of stigma length and ovary length, so the length of these parts of the gynoecium seems to be independent of the whole gynoecium length.

Table 4. Correlation coefficients in thrum and pin morphs

	length of gynoecium		length of stigma		length of ovary	
	thrum	pin	thrum	pin	thrum	pin
length of style	**0.91**	**0.97**				
length of stigma	0.28	0.10				
length of ovary	0.26	0.07	0.08	–0.07		
width of stigma			0.32	0.31		
width of ovary					**0.63**	**0.64**

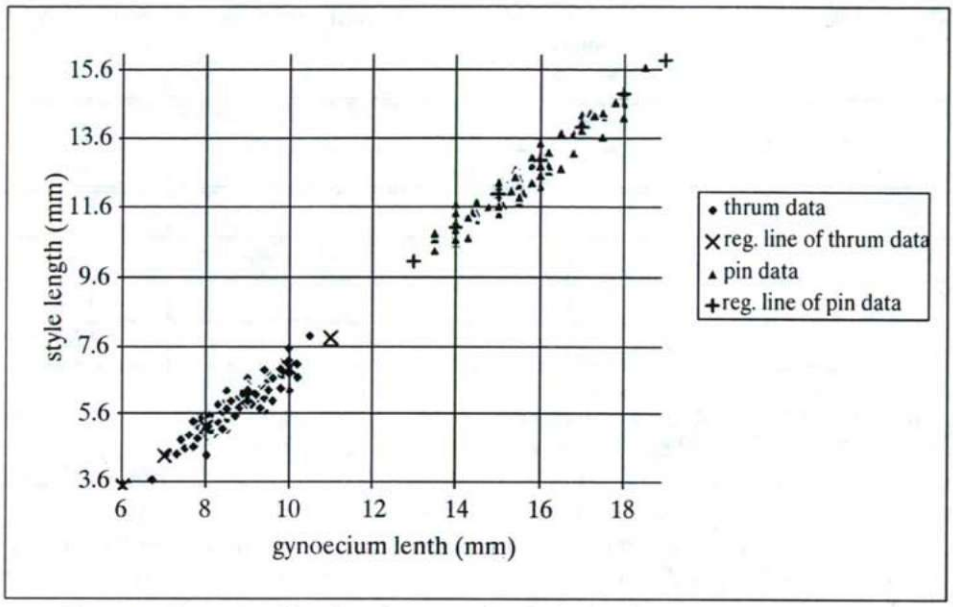

Figure 1. Gynoecium length as function of style lenght (reg. line = regression line)

There is no correlation between the stigma length and the ovary length at all, the correlation coefficients are practically zero, and the regression lines are almost parallel with the X-axis. But a slight connection can be revealed between the length and the width of the stigma and the ovary. In the case of ovary length and width the relation is stronger: the correlation coefficients are about 0.6 and the regression lines are quite steep.

Number and size of ovules

Mean, standard deviation and range of number and size of ovules are summarised in Table 5. The mean and the standard deviation of the number of ovules per ovary are equal in the two morphs, there is no significant difference between them. The frequency distribution diagrams of the morphs are also very similar, the peaks of the thrum and pin data are in the category of 60–70 pieces with 31% and 38% of all the cases, respectively. The number of ovules does not correlate with the ovary length at all, but it seems to be slightly connected with the ovary width (thrum correlation coefficient is 0.35 and pin one is 0.39).

Table 5. Mean, standard deviation and range of ovule number and ovule length
(St. dev. = standard deviation, N= number of cases)

	Number of ovules per ovary		Length of ovules (μm)	
	thrum	pin	thrum	pin
Mean	55	54	399	432
St. dev.	13	12	46	67
Range	56	75	271	365
N	120	117	525	479

In contrast to the number of ovules the length of them is significantly different in the two morphs at $P \leq 0.001$: the pin ovules are slightly larger than the thrum ones. The standard deviation and the range are also bigger in the pin morph than in the thrum one, similarly to most of the examined features. The frequency distribution diagrams also show the most general behaviour of the two morphs: the thrum data have a characteristic peak, while the pin diagram is much flatter (Fig. 2).

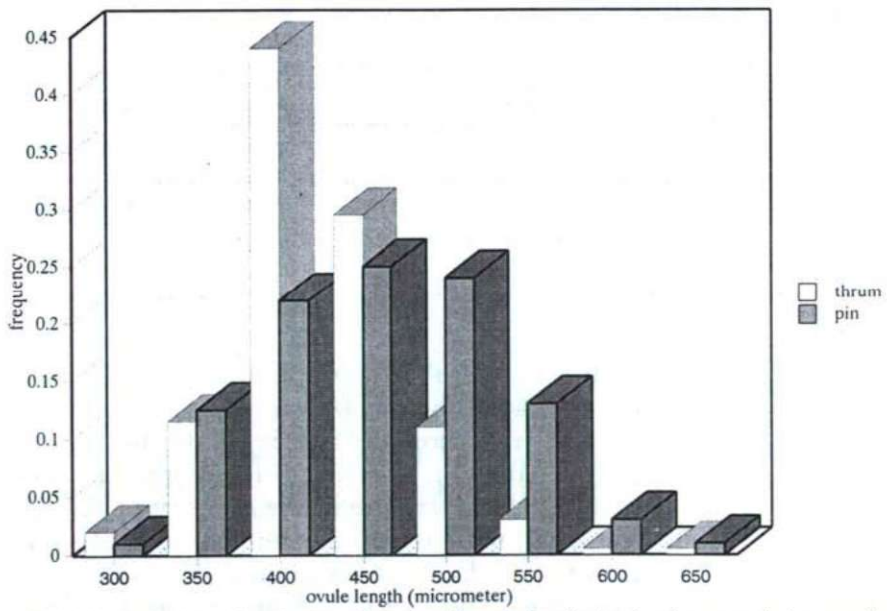

Figure 2. Frequency distribution diagram of ovule lenght in the thrum and pin morphs

Conclusions

The most characteristic manifestation of reciprocal herkogamy is the occurence of two types of gynoecium: thrum one with short style and pin one with long style. According to our results the gynoeciums can distinguish unambiguously in this respect and the pin:thrum ratio is 2:1.

Difference in the stigma size between the two morphs is reported in some *Primula* species and two types have been described: in some species the pin stigma is larger than the thrum one (HESLOP-HARRISON *et al.*, 1981), whereas in other species the ratio is opposite (PANDEY and TROUGHTON, 1974). Our results revealed that pin and thrum stigma is significantly different at $P \leq 0.001$ by both length and width confirming that the pin stigma is longer and wider than the thrum one.

The ovule size is a rarely examined feature of heterostylous species, so our observation that the largest length of the pin and thrum ovules is significantly different at $P \leq 0.001$ is a new result.

For style length, stigma size and ovule length where significant difference was found between the morphs the standard deviation and the range of values are larger in the pin morph than in the thrum one which means that variability of these pin traits is more considerable than that of thrum ones. For these features the frequency distribution diagrams also reveal characteristic differences between the morphs: in thrum diagrams there is a definite peak with a high frequency, while the pin diagrams are flat with several similar frequencies in the middle of the range.

Ovary length, ovary width and ovule number per ovary do not differ between the two morphs, their variability is low and the frequency distribution diagrams of the pin and thrum data are almost the same, so these characters seem to be enormously conservative.

Correlation studies show that the length of the gynoecium is strictly determined by the style length, but apart from this strong connection there is no correlation between the size of the gynoecium parts.

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REPRODUCTIVE STRATEGIES OF *POA BULBOSA* L. VAR. *VIVIPARA* KOEL. FROM DISTURBED GRASSES OF EAST-HUNGARY

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Abstract

The reproductive strategies of a wide-spread xerophylous species, *Poa bulbosa* L. var. vivipara KOEL. was studied on samples collected from different populations of sandy and alkaline pastures between 1993 and 1996. High viviparism was observed in each population, and a low ratio of fertile flowers. The dominance of apomictic ways in proliferation was proved in the sprouting experiments. The year of more precipitation promoted the production of bulbils and caryopses. The population of alkaline soil showed more stability in these features during the four years studied.

Key words: bulbs, bulbils, caryopses, spikelets, sterility, sprouting experiments

Introduction

Arid and semiarid pasture grasses are important entities on sandy and salty soil in East Hungary. Besides their economical importance they preserve the element of the original ancient vegetation. Studying the dynamics and key species helps us to maintain and preserve them (FEKETE *et al.*, 1988; KÖRMÖCZI, 1991; MATUS and TÓTHMÉRÉSZ, 1991a, 1991c, 1994; NAGY *et al.*, 1990, 1991; PRÉCSÉNYI *et al.*, 1990). *Poa bulbosa* L. var. vivipara KOEL. has been an increasing role in these grasses, since the climate changed drier in the Carpathian basin since the end of 1970s. The species has an intensive distribution in the middle and south parts of Eurasia, and in North Africa too (HEGI, 1906; MEUSEL, 1965). It is a Festuco-Brometea species according to the Hungarian coenosystem (SOÓ, 1973) preferring the arid or semiarid sandy habitats, but also occurring in alkaline grasslands. In SIMON system

(1988) it is a natural disturbance tolerant species. The wide spread of the species is attributed to the wide-scale collection of ecotypes and the varied reproductive strategies (WYCHERLEY, 1953, 1954; YOUNGER, 1960; CHAPMAN, 1990). Underground bulbs survive both the extra cold and hot. In the inflorescence, besides the caryopses, bulbils can grow instead of flowers to avoid the uncertain factors in sexual reproduction. The phenomenon of the development of vegetative shoots among the reproductive organs belongs to viviparism in wide sense (LATTING, 1972; HEIDE, 1988). It is an apomictic (clonal) way of reproduction just as the production of bulbs in tussocks. The aim of our study is to get data on phenotypic plasticity and the reproductive strategies of *Poa bulbosa* populations growing in the surroundings of Debrecen (East-Hungary).

Sites and Methods

In the surrounding of the town Debrecen five *Festucetum pseudovinae* grass communities were studied seasonally between 1994 and 1996. Four of the five study areas (in the boundary of villages Bagamér, Bátorliget, Debrecen and Penészlek) are on sandy soil, and the fifth is on alkaline soil in Hortobágy National Park. Each one represents a different stage of succession and is under different degree of disturbance (grazing and trampling). Parallel to the phytocoenological observations *Poa bulbosa* individuals were collected for morphological studies. The study units were 100–100 mature reproductive shoots from each site, harvested in Junes between 1993 and 1996.

The length of shoots holding panicles, number of nodes on them, length and the largest width of the panicles and the number of branches and spikelets were studied and discussed in an other paper (M. HAMVAS *et al.*, 1997).

Considering the reproductive strategy, the composition of the spikelets, the number of the sterile and fertile flowers and their ratio to the bulbils were investigated. All data were calculated from 100–100 repetitions.

The bulbs for sprouting were collected in summer of 1996, in latent state. The sprouting study of the bulbs, bulbils and caryopses were carried out in the end of September also after two week treatments of +4°C. The propagules were sprouted on wet paper both on light and dark.

Results

The life cycle of Poa bulbosa L. var. vivipara Koel.

The renewing of bulbous blue grass tussocks and the germination of the caryopses can begin from late September, depending on the precipitation (Fig. 1). They become persistent to survive the severity of the winter. Depending on the weather, they can sprout again in the end of January and produce strong tussocks with new intravaginal bulbs for March. The generative shoots can appear already at the beginning of April. They start to flower at the beginning of May. Mature (yellow) panicles, bulbils and caryopses can be collected till the middle of June.

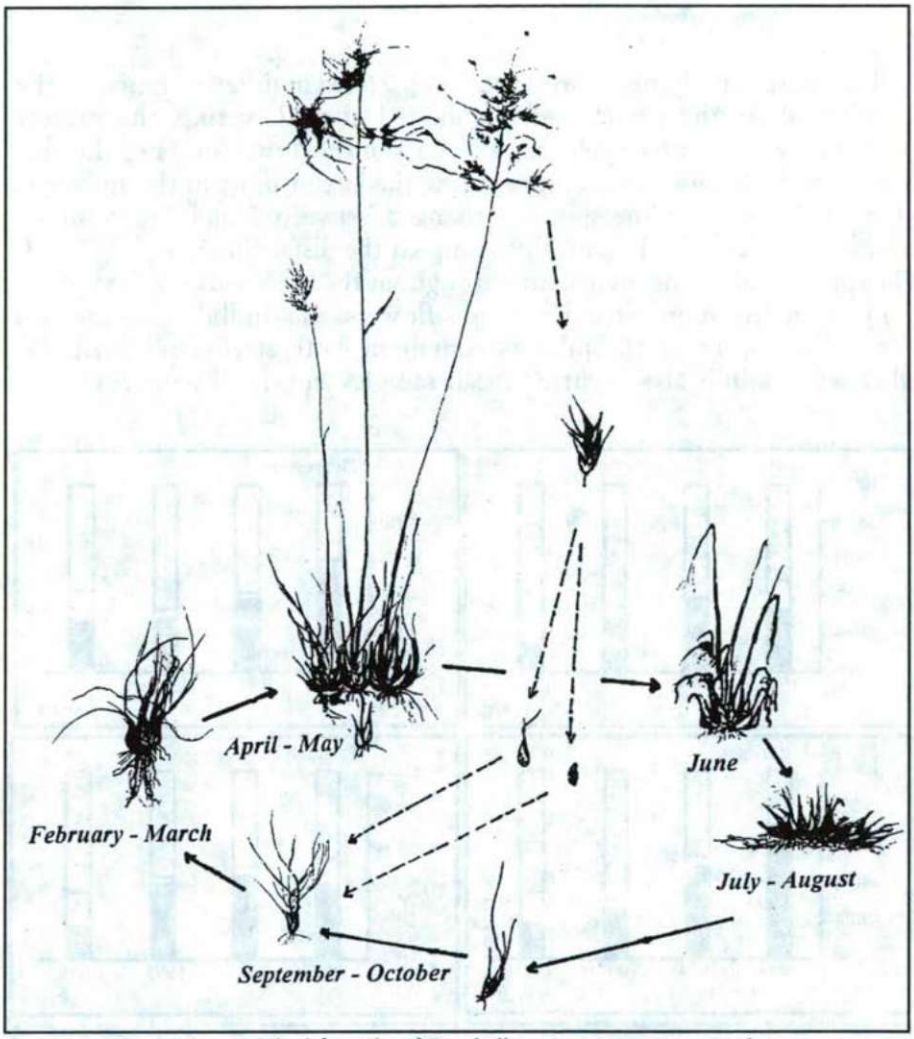

Figure 1. The life cycle of *Poa bulbosa L. var. vivipara Koel.*

Both the bulbs and the bulbils can grow separately on long internodes too: The case of bulbs signs that extravaginal growing can occur too. The bulbils or a bulbil of distal position on the spikelet axes also can emerge rarely from the spikelet, giving the panicle a tousled appearance. The shoots of the species spend the period of drought in the form of dry latent bulbs. On pastures lives-stock promotes the spreading of the bulbs by trampling. The light caryopses can serve the long distance spreading by wind.

Composition of the spikelets

Bulbils, sterile and fertile flowers can be registered in different ratios on the axes of a spikelets. As the panicles were collected after flowering, the presence or absence of caryopses differs well the two types of the flower site. For this time the bulbils are well developed too, representing the largest units in the spikelets. The number of the units in the spikelets changed between 3 and 11, in an average spikelet it is 5. The bulbils generally occupied the distal sites.

The species had strong viviparism throughout the four years on all studied areas (Fig. 2). Spikelets dominated by sterile flowers and bulbils characterised the samples (47–94%, Table 1). Spikelets containing both sterile and fertile flowers together with bulbils also occurred in all samples and in all years (but less then

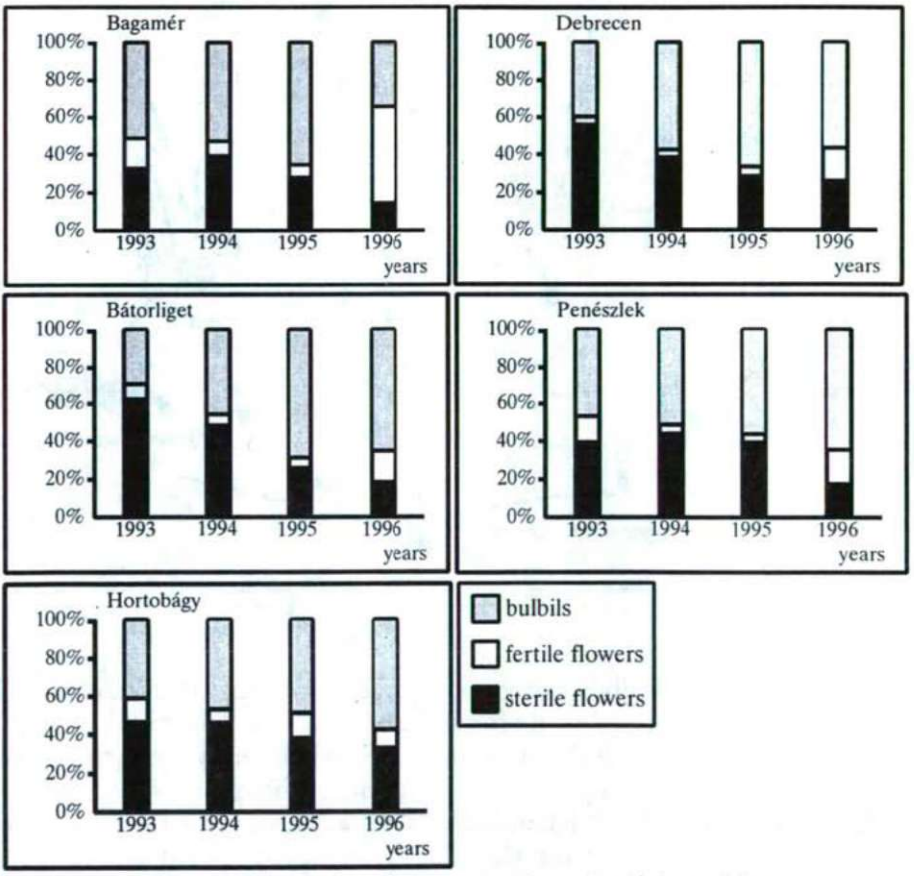

Figure 2. Distribution of the propagation units of the panicles in the samples of the different sites between 1993–1996

17%). Spikelets, which did not serve the reproduction any ways (containing only sterile flowers), were not at all in the studied sets (Table 1).

Table 1. Distribution of the spikelet types in the panicles of the studied sites and years (%)

Distribution of spikelet types in the panicle, %	sterile fl., bulbils —	— bulbils, fertile fl.	sterile fl., bulbils, fertile fl.	— bulbils —	sterile fl., — fertile fl.	fertile fl. — —
Bagamér						
1993	70	16	14	0	0	0
1994	78	5	17	0	0	0
1995	83	10	6	1	0	0
1996	48	7	9	0	4	32
Bátorliget						
1993	79	2	11	7	0	1
1994	91	0	9	0	0	0
1995	84	0	9	6	0	1
1996	47	18	14	18	1	2
Debrecen						
1993	93	0	7	0	0	0
1994	94	5	1	0	0	0
1995	74	0	5	19	1	1
1996	50	8	16	18	1	7
Penészlek						
1993	71	25	1	0	0	3
1994	94	1	5	0	0	0
1995	94	2	4	0	0	0
1996	51	13	8	19	0	9
Hortobágy						
1993	79	9	12	0	0	0
1994	89	0	11	0	0	0
1995	87	3	10	0	0	0
1996	75	1	10	11	2	1

Variations between the years and the sites

In 1996, when more precipitation fell just before flowering than in the previous years, the percentage of caryopses in the spikelets increased, except the Hortobágy

sample (Fig. 2). Parallel to this tendency the number of the spikelet types increased too (Fig. 3). Homogeneous spikelets containing only fertile flowers or only bulbils appeared, which were rare or absent earlier. The changes in this feature between the years were the least in the sampling of alkaline site (Fig. 2, 3).

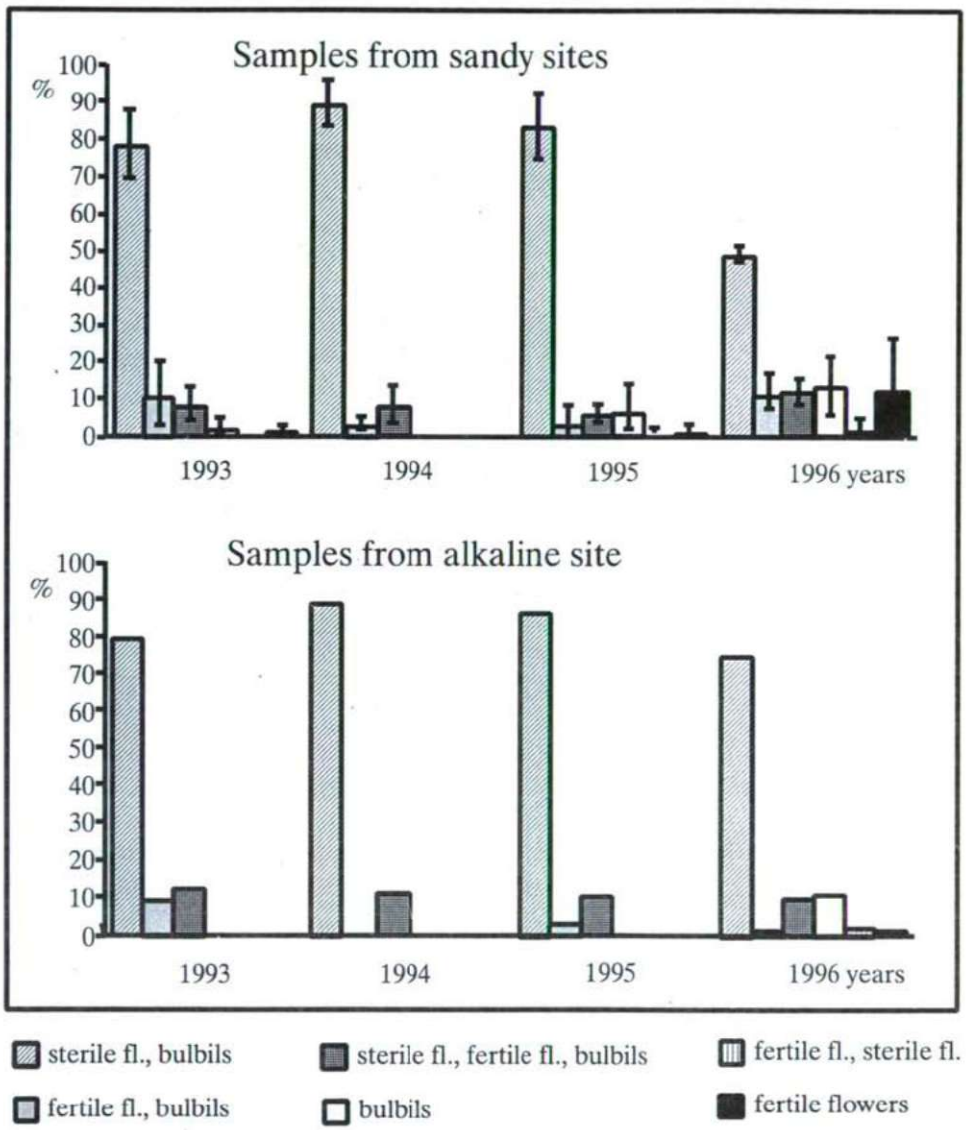

Figure 3. Spikelet types of the samplings from sandy grasses (the average of the five sites) and from the alkaline grass during the four years

The species proved to be flexible in different phytosociological surroundings. Studying the sandy sites, they showed certain deviations but without any trend (Table 1, Fig. 2). We found that the sterility is lower there (35% to 42%), and the rate of the bulbils is higher (50%–58%), taking the average data of the years for each site, comparing to the data of alkaline site. The values are 42% and 48%, respectively in the later case, for the sample of Hortobágy. Therefore the production of caryopses in the average of the years in an average panicle is almost the same (about 10%) on the different sites (and on the two soil types, Fig. 2).

Sprouting capacity of the proliferation units

The bulbs sprouted well in every sample. The cold treatment did not influenced the sprouting rate, it was above 94% in the samples of all sites. There was no significant difference between sprouting in light and dark. It corresponds with the field experience, that the bulbs rooted just above the soil surface.

Considering the propagules from the panicles, their further vitality are much less then that of to the bulbs. The sprouting of the bulbils and the germination of the caryopses varied without any trends in the samples of different sites and after different treatments. The percentages always remained under 20%. The sprouting of the propagules occured with few days quicker without cold treatment (after 6 days were no new roots appeared). It suggests that the wet has greater role in the autumn renewing than the cold after about a few-month dormancy. Considering the above results and the rate of the bulbils and caryopses in the panicles (the first is more than 10-fold higher), the bulbils are the main agents after the bulbs in the reproduction. Caryopses of low weight can serve only the long distance spreading of the species.

Summary

Poa bulbosa L. var. vivipara KOEL. appears to be a key factor in the maintenance of degraded pastures in arid climate and habitats. The paper describes the reproductive behaviour of the species in different plant communities in East Hungary. On the studied sites (on 5 pastures) the latent bulbs proved to be the main strategy. The bulb vitality was very high. The composition of the spikelets in the panicles is varied. All spikelets served somehow the reproduction. The number of bulbils almost always exceeded that of the sterile flowers and much more did that of the caryopses. Higher precipitation before flowering resulted in increase of spikelets types and in increase of the caryopsis number. The vitality of bulbils and caryopses was much more lower than that of bulbs. Considering the high bulbil-caryopsis ratio, the bulbils have yet great role in reproduction, after the bulbs.

The studied features on the reproductive shoots did not prove ecotypes among the populations of the different sandy sampling sites. The samples from alkaline soil showed smaller deviation from the sandy sites with higher sterility and less bulbils and with its stability during the years. Smaller data for the panicles and thicker and harder tissues (M. HAMVAS *et al.*, 1997) also suggests some segregation.

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MECHANISMS OF GRAVITY PERCEPTION AND TRANSDUCTION IN PLANTS: RESULTS OF GROUND AND SPACE EXPERIMENTS

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Abstract

Gravitropism is directed growth of a plant or plant organ in response to gravity and can be divided into the following temporal sequence: the perception, transduction, and response phases. This paper is a review of the research on gravity perception and transduction mechanisms in plants. The evidence for the two standing theories of perception, the starch statolith and protoplast pressure models, is presented along with a new theory (the statolith pressure) that attempts to synthesize both models. This review considers both ground-based and spaceflight research and ends with results of recent flight experiments that address these issues.

Key words: gravitropism, statolith, *Arabidopsis,* spaceflight experiments, starch-deficient mutant.

Introduction

Plants are capable of very exquisite responses to their environment, and the tropisms provide an excellent example of this phenomenon. A tropism is a directed growth response to an external stimulus such as light (phototropism), touch (thigmotropism), or gravity (gravitropism, formerly known as geotropism). This paper provides a review of plant gravitropism with a focus on the earlier events of the gravitropism pathway, namely the perception and transduction phases. Other recent review articles on this subject include those by SALISBURY, 1993; BALUSKA and HASENSTEIN, 1997; SACK, 1997.

In terms of sensory physiology, gravitropism is the directed growth of a plant or plant organ in response to the force of gravity. The stages of gravitropism in

plants can be divided into: perception, transduction, and response (EVANS *et al.*, 1986; SALISBURY, 1993). In roots, gravity perception is hypothesized to occur in the rootcap region, and the response (differential growth) occurs in the zone of elongation (SACK, 1991). In shoots, the endodermis appears to be the site of perception (KISS and SACK, 1990; FUKAKI *et al.*, 1998). In higher plants, there is a cellular and spatial separation between perception and response, and the signal must be transmitted over a relatively large distance (i.e. many cell layers). In certain lower plant groups, all phases of gravitropism occur in a single cell (SIEVERS *et al.*, 1996; KISS, 1997).

Hypotheses for Gravity Perception

Until recently, there have been two principal models for plant gravity perception (SACK, 1997). First, there is the **starch statolith hypothesis**, which proposes that perception is mediated by the interaction of dense organelles (termed statoliths) with other cytoplasmic structures. In higher plants, the statoliths appear to be amyloplasts in specialized cells while other types of sedimenting particles have been found in algae. The statolith model has been supported by work from our group and others (e.g. KISS *et al.*, 1989, 1996, 1997; VITHA *et al.*, 1998). An alternate model is the **protoplast pressure hypothesis**, which has been advocated by Wayne and co-workers (e.g. WAYNE *et al.*, 1992; STAVES *et al.*, 1995, 1997a, 1997b). From a thermodynamic perspective, calculations show that either model is feasible (HASENSTEIN, 1999). However, recently PERBAL (1999) has attempted to reconcile these two models and proposed that both the protoplast and statoliths play a role in perception in his **statolith pressure theory**.

The starch statolith hypothesis has been discussed in numerous older reviews (VOLKMANN and SIEVERS, 1979; EVANS *et al.*, 1986) and more recent publications (SALISBURY, 1993; SACK, 1997). The main lines of evidence for a statolith-based model are presented in these papers and can be summarized as follows. (1) In stems of several plant species, the response to gravity is correlated with the location and extent of amyloplast sedimentation. (2) *Arabidopsis* mutants lacking an endodermis in stems are agravitropic (FUKAKI *et al.*, 1998). (3) During the regeneration of a rootcap following its removal, the ability to perceive gravity was restored when the cap was reformed and new amyloplasts sedimented. (4) Reduced-starch and starchless mutants of *Arabidopsis* and *Nicotiana* have vigorous growth but are less sensitive to gravity in ground-based (KISS *et al.*, 1997; VITHA *et al.*, 1998) and in spaceflight studies (KISS *et al.*, 1998, 1999). (5) Magnetophoretic displacement of amyloplasts induced curvature in roots. (6) Rapid changes in membrane potential occur in central rootcap (columella cells), but not in other cells of the root, following gravistimulation by reorientation.

However, there also is a body of evidence for the protoplast pressure hypothesis (reviewed in SACK, 1997, and STAVES *et al.*, 1997a, 1997b). (1) Sedimenting particles are absent in many gravitatic unicells such as *Euglena* and in internodal cells of the alga *Chara*. (2) The density of external medium alters the gravity-dependent cytoplasmic streaming in internodal cells of *Chara*. (3) There is a possibility (based on inhibitor studies) that integrin-like molecules sense changes in protoplast pressure. (4) The density of external medium also appears to alter gravitropism in roots of higher plant (rice; STAVES *et al.*, 1997b). However, as stated above, a new proposal to integrate both the statolith model and the protoplast pressure model has been made by PERBAL (1999). FURTHERMORE, BARLOW (1995) and SACK (1997) have proposed that several types of gravity sensing exist in plants, although this view has been discounted by the Wayne group (STAVES *et al.*, 1997b).

If statoliths play a role in gravity perception, then there are several possibilities for their mode of action. One view is that sedimentation or (relatively) larger scale movement is needed for statolith function (VOLKMANN and SIEVERS, 1979). In this case, starchless mutants would perceive gravity by another mechanism since it appears that starchless plastids do not move upon reorientation of roots (CASPAR and PICKARD, 1989; KISS *et al.*, 1989; MACCLEERY and KISS, 1997). Another view is that statoliths act through a pressure mechanism and that statolith sedimentation is not need (NICK *et al.*, 1997).

Potential Role of the Cytoskeleton in Gravitropic Signal Transduction

Following the perception of gravity, how is the signal transduced so that a response will occur? Many researchers have suggested the cytoskeleton, both microfilaments and microtubules, plays a role in the perception/transduction phase (BALUSKA and HASENSTEIN, 1997). One of the earliest proposals for microtubular involvement in gravitropism in plants was by FRIEDRICH and HERTEL (1973). Later studies seem to focus more the microfilament cytoskeleton by a combination of sophisticated immunolocalization studies and by inhibitor studies with cytochalasins (HENSEL, 1985; SIEVERS *et al.*, 1989, 1991). Spaceflight experiments also have examined the interrelationships between statoliths and microfilaments (e.g. BUCHEN *et al.*, 1993). In addition, integrin-like molecules (which may link the microfilaments to the extracellular matrix) in plants have been proposed to be involved in gravitropic signal transduction (WAYNE *et al.*, 1992; KATEMBE *et al.*, 1997).

However, despite the availability of some of the above cited correlative evidence, researchers are now calling into question the role of both microtubules and microfilaments in perception/transduction mechanisms of gravitropism. For instance, microtubules cannot be identified in the tip region of gravitropic *Chara* rhizoids (BRAUN and SIEVERS, 1994). Similarly, microfilament bundles cannot be

localized to the central root cap cells of higher plant roots (BALUSKA and HASENSTEIN, 1997). Cytochalasin was shown, in fact, not to inhibit gravitropism in roots of three species of higher plants (STAVES *et al.*, 1997a). In addition, in a recent spaceflight experiment, roots that were treated with cytochalasin and then subject to centrifugation curved *faster* than the controls (PERBAL, 1999)! However, although microtubule arrays and microfilament bundles (i.e., F-actin cables) have been ruled out by most workers, it is possible that single or small groups of microfilaments can play a role in transduction (BALUSKA and HASENSTEIN, 1997).

Starch-Deficient Mutants — Importance in Gravitropism Research

Some of the strongest evidence that amyloplasts function as statoliths comes from our research with starchless mutants of *Arabidopsis* (KISS *et al.*, 1989; SACK and KISS, 1989). Based on detailed studies of the kinetics of gravitropic curvature, the conclusion was that wild-type (WT) roots (with a full complement of starch) are more sensitive to gravity than starchless roots. For example, the presentation time (a measurement of gravitropic sensitivity; JOHNSSON and PICKARD, 1979) was 0.4 minutes for the normal WT and 1.3 minutes for the starchless (TC7) mutant. Other research with *Nicotiana* has shown that roots and hypocotyls of a low starch mutant were much less sensitive to gravity compared to WT roots and hypocotyls (KISS and SACK 1989, 1990; VITHA *et al.*, 1998). In all of these studies, the greatest difference in gravitropic sensitivity was found at threshold „doses" of gravity that were estimated by comparisons of presentation times and perception times.

This work was extended in studies of two reduced-starch mutants and an independently-isolated starchless mutant of *Arabidopsis*, which demonstrated that the degree of graviresponsiveness is proportional to the total mass of plastids per cell (KISS *et al.*, 1996, 1997; WEISE and KISS, 1997). These „intermediate" mutants have 50 and 61% of the WT starch in columella cells of the root cap (KISS et al., 1996) and also are starch-deficient in their hypocotyls (KISS *et al.*, 1997). However, as indicated by STAVES *et al.* (1997b), „while these data are consistent with the statolith theory, they do not discriminate between the gravitational pressure and statolith theories for gravisensing."

Our Spaceflight Experiments with Arabidopsis

Thus, despite extensive study for almost a century, the starch-statolith theory remains controversial, at least to some investigators (HENSEL, 1989). A major reason for this controversy is the intrinsic difficulty of estimating gravitropic sensitivity in a 1-*g* environment. Since gravity is a constant and ubiquitous force in ground-based studies, the principal method to estimate gravitropic sensitivity (e.g.

presentation time) in plants involves the use of clinostats (e.g. KISS *et al.*, 1989, 1996), which have their own inherent limitations. However, we had the opportunity for a spaceflight project to study gravitropic sensitivity in WT and starch-deficient *Arabidopsis* (two reduced-starch and one starchless mutant) in microgravity by using an in-flight 1-*g* centrifuge to apply limited amount of gravitational forces so that thresholds could be estimated.

In 1995, our space project was selected by the National Aeronautics and Space Administration (NASA) and the European Space Agency (ESA) for definition and development as part of a Space Shuttle payload called Biorack (BRILLOUET and BRINCKMANN, 1997; KATEMBE *et al.*, 1998). The Biorack module is a multiuser facility which serves as a small laboratory for studying cell and developmental biology in unicellular organisms, plants, and small invertebrates (MANIERI *et al.*, 1996). This facility consists of two incubators (each with two variable-*g* centrifuges), a glovebox, and, during spaceflight missions, a duplicate Biorack unit exists on the ground as a control.

Our first spaceflight project, called PREPLASTID, was a smaller-scale experiment on Space Shuttle STS-81 that flew in January 1997 and was designed to assess the growth/developmental characteristics of the *Arabidopsis* plants (KISS *et al.*, 1998). Based on the PREPLASTID results, we optimized our procedures for the larger-scale PLASTID experiment on STS-84 that flew in May 1997 (KISS *et al.*, 1999).

The most significant result of these spaceflight experiments is that WT hypocotyls of microgravity-grown seedlings had the strongest response to stimuli provided by the 1-*g* centrifuge while hypocotyls of the starchless mutant did not. The reduced starch mutants exhibited a response intermediate between the WT and the starchless strain.

What was unexpected in our flight studies was the small magnitude of curvature that resulted after the unilateral gravitational stimulus provided by the centrifuge (KISS *et al.*, 1998, 1999). Based on our ground studies (KISS *et al.*, 1996, 1997), we predicted a greater magnitude of curvature for all four strains of *Arabidopsis*. One possible interpretation is that hypocotyls of seedlings grown on the ground are more sensitive to gravity than are those of the microgravity-grown seedlings. These results differ from studies of roots, which have been shown to be more sensitive in microgravity compared to a 1-*g* environment (PERBAL and DRISS-ÉCOLE, 1994; VOLKMANN and TEWINKEL, 1996; PERBAL *et al.*, 1997). However, since the space grown plants were smaller than the ground controls (KISS *et al.*, 1998), the difference in gravitropic sensitivity may be more related to developmental stage of the seedlings rather than simply differences in sensitivity between roots and hypocotyls.

This experiment on gravitropic sensitivity was performed the „right way" in that brief gravitational stimuli were provided, and the seedlings were allowed to

express the response without further unilateral gravity stimuli. Thus, the complications of ground-based experiments performed with clinostats were avoided (SALISBURY, 1993), and the unique environment of microgravity was used to help answer basic questions about biological phenomena (DUTCHER *et al.*, 1994). The results of PLASTID and PREPLASTID support previous ground-based studies of these and other mutants which demonstrate a positive correlation with increasing amounts of starch and increasing sensitivity to gravity. Taken together, the ground and space research are strongly supportive of a statolith-based model for plant gravity perception but still do not exclude the protoplast pressure hypothesis. Further ground-based and spaceflight research should help us to distinguish among the competing hypotheses for graviperception mechanisms in plants.

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VARIATION IN DICOT C_4 TYPE LEAF ANATOMY IN THE HUNGARIAN FLORA

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Abstract

A considerable variation in Kranz anatomy was encountered in the Hungarian angiosperm flora, we utilised the examination results of in total 298 species. The anatomical structure of the leaves has been examined by using light microscope. Altogether we found 23 C_4 dicot species in the *Amaranthaceae* (10), *Chenopodiaceae* (8), *Euphorbiaceae* (3), *Portulacaceae* (1) and *Zygophyllaceae* (1) families. It is demonstrated that there are possibly two different types of Kranz anatomy. While in the typical Kranz syndrome each vascular bundle is surrounded by a bundle sheath, these non typical types contain a „collective" Kranz sheath organised around multiple bundles in the leaf. The *Chenopodiaceae* showed the greatest variation with three structural types for eight species, and with the most unusual leaf structural variants (i.e. kochioid and salsoloid types). The non-typical Kranz structures mainly occur among plants of the most extreme habitats (semidesert-like saline and sand grasslands). The observed anatomical variation reflects the poliphyletic origin of the C_4 photosynthesis and the diversity of biotopes inhabited by C_4 plants.

Key words: Amaranthoid type, Atriplicoid type, Euphorboid type, Kochioid type, non-typical Kranz anatomy, Salsoloid type, typical Kranz anatomy

Introduction

Since its discovery in the mid-sixtes, the C_4 photosynthetic pathway has been continuously staying in the centre of scientific attention. Examining corn leaves HABERLANDT (1918) recognized a sheath of vast, thin-walled cells around vascular bundles, which was surrounded by radially organised palisade-like mesophyll cells. He termed this leaf structure Kranz anatomy. During further physiological studies

HATCH (1976, 1987) discovered that the C_4 pathway of photosynthesis is associated with this structure.

C_4 plants occur in more than 200 genera of 18 angiosperm families, ca. 1000 species (DOWNTON, 1975; WATSON and DALLWITZ, 1993) fix carbon via the C_4 pathway (HATTERSLEY, 1987; RAGHAVENDRA and DAS, 1978). During this work a remarkable variation in the anatomical structure had been observed and classifications of Kranz anatomy types was elaborated (e.g. CAROLIN et al., 1975; HATTERSLEY and WATSON, 1976). Nevertheless, all anatomical variants share one fundamental feature (DENGLER et al., 1985); i.e. the leaf chlorenchyma is always differentiated into two functional units: the mesophyll and the bundle sheath. By this arrangement the two major steps of C_4 metabolism becomes spatially separated within the leaf. Namely, CO_2 is fixed into malate or aspartate in the mesophyll (primary carbon assimilation /PCA/ tissue), then the acids are transported into the bundle sheath (photosynthetic carbon reduction /PCR/ tissue). Here they are C_4 acid decarboxilation and the resulting CO_2 enters the Calvin cycle and finally starch is formed. Further research revealed biochemical subtypes of the C_4 metabolism (see HATCH, 1987 for review), and relationships have been found between leaf anatomy and biochemical subtypes (DOWNTON, 1975; DOWNTON et al., 1969; EDWARDS and HUBER, 1981; GAMALEJ and VOZNYESZENSZKAJA, 1986; GUTIERREZ et al., 1974). According to the primary product of CO_2 fixation, the decarboxylation enzyme and the anatomy and ultrastructure of bundle sheath cells in the case of dicot plants the following C_4 subtypes have been distinguished (GUTIERREZ et al., 1974; HATTERSLEY, 1984; HATCH, 1987; VOZNYESZENSZKAJA and GAMALEJ, 1986).

1. NADP-ME. The first product of CO_2 fixation is malate, that is decarboxylated in the bundle sheath by NADP malic enzyme (NADP-ME). Bundle sheath chloroplasts lack grana and are centripetally arranged. Mesophyll chloroplasts contain grana.
2. NAD-ME. Carbon dioxide is fixed into aspartate, the decarboxylating enzyme is NAD malic enzyme (NAD-ME). Bundle sheath chloroplasts are granal and centripetally positioned, while mesophyll chloroplasts lack grana.

The Kranz anatomy has been studied particularly in detail in the *Chenopodiaceae* (CAROLIN et al., 1975; DOWNTON et al., 1969; DENGLER et al., 1995; KNAPP-ZINN, 1984; LIU and DENGLER, 1994; PATRIGNANI et al., 1993; PJANKOV and VAHRUZSEVA, 1989; OSMOND et al., 1980; VASZILEVSZKAJA and BUTNIK, 1981; WELKIE and CALDWELL, 1970).

The screening of the Hungarian angiosperm flora for the occurrence of C_4 plants has been completed recently (KALAPOS, 1991; KALAPOS et al., 1997; NYAKAS, 1991, 1992; NYAKAS and KALAPOS, 1996). During this work leaf anatomy was one of the traits used for the determination of the photosynthetic pathway type of plants. This paper summarizes the variation of Kranz anatomy in dicot plants encountered during the survey.

Table 1. Dicot C_4-species in Hungarian Angiosperm flora.

Where literature data are used reference to source is given in superscript. Life forms: Th= therophytes, H= hemicryptophytes, Ch= chamaephytes, N= nanophanerophytes, (Phytogeographical and life forms classification of species follows of SIMON 1992). Literature sources: 1. MATEU 1993, 2. WELKIE et al. 1970

Anatomical structure	Taxon	Floristic element	Life forms
Typical Kranz anatomy			
Amaranthoid type			
	Amaranthaceae		
[1,2]Amaranthus albus L.		adventive	Th
A. blitoides S. Watson		adventive	Th
[1,2]A. bouchonii Thell.		adventive	Th
A. chlorostachys Willd.		cosmopolitan	Th
[1,2]A. crispus (Lesp. et Théven) N. Terrac		adventive	Th
A. deflexus L.		adventive	H
[1,2]A. graecizans L.		south-eurasian	Th
[1,2]A. lividus L.		cosmopolitan	Th
A. patulus Bert.		adventive	Th
A. retroflexus L.		cosmopolitan	Th
	Zygophyllaceae		
Tribulus terrestris L.		cosmopolitan	Th
Euphorboid type			
	Euphorbiaceae		
Euphorbia humifusa Willd.		adventive	Th
E. maculata L.		adventive	Th
E. nutans Lag.		adventive	Th
	Portulacaceae		
Portulaca oleracea L.		cosmopolitan	Th
Atriplicoid type			
	Chenopodiaceae		
Atriplex rosea L.		south-eurasian	Th
A. tatarica L.		eurasian-(med)	Th
Non typical Kranz anatomy			
Kochioid type			
Kochia laniflora (Gmel.) Borb.		eurasian	Th
K. prostrata (L.) Schrad.		eurasian-(med)	Ch-N
K. scoparia (L.) Schrad.		eurasian	Th
Salsoloid type			Th
Salsola kali L.		eurasian-(med)	Th
S. soda L.		eurasian-(med)	Th
Camphorosma annua Pall.		ponto-pannonic	

Materials and Methods

C_4 plants occur in more than 200 genera of 18 angiosperm families (15 families in dicot and 3 in monocot), ca. 1000 species (DOWNTON, 1975; WATSON and DALLWITZ, 1993) fix carbon via the C_4 pathway. In search for dicot C_4 plants in Hungarian flora 66 species representing 15 families have been examined. We used additional literature data, if available, with the source always indicated. In our test, mostly those families were screened where the occurence of C_4 species had reported earlier (CAROLIN et al., 1975; DENGLER et al., 1995; LIU and DENGLER, 1994; MATEU, 1993). Nomenclature follows DAHLGREN et al. (1985) and SIMON (1992).

The leaf anatomy was studied on cross section of leaf blades (mature stem leaves) and wherever possible fresh plant material was used to prepare sections for examination with the light microscope. This material fixed with in a 1:1:1 mixture of abs. alcohol, glycerine and distilled water. Sections were cut 10–12 μm thick and the samples were either stained with azur-hematoxilin or treated with the lugol solution to identify the localisation of starch accumulation within the chlorenchyma. Light microscopic pictures of various enlargement were recorded by using a computer image processing system and stored on CD-ROM.

Results and Discussion

Altogether we found wild 23 C_4 dicot species in the *Amaranthaceae* (10), *Chenopodiaceae* (8), *Euphorbiaceae* (3), *Portulacaceae* (1) and *Zygophyllaceae* (1) families (Table 1.). These species in Hungary are mainly cosmopolitan plants, or naturalised aliens or weeds, only few of them are native. They are predominantly annual species inhabit dry grasslands, saline areas, temporally exposed riverbeds and disturbed sites (COLLINS and JONES, 1985; KALAPOS, 1991; KALAPOS et al., 1997; NYAKAS, 1992). Hungary's geographical situation and the diversity of its natural habitats can explain that its flora is richer in C_4 species than of other temperate — zone countries.

The leaf anatomy of dicot C_4 plants is more diversified than of the monocot ones. In addition to the so-called typical Kranz anatomy (Kranz-rosette mesophyll, HABERLANDT, 1918) characteristic to grasses, several non-typical arrangements occur. While in the typical Kranz syndrome each vascular bundle is surrounded by a bundle sheath, these non typical types contain a „collective" Kranz sheath organised around multiple bundles in the leaf, which is surrounded by the PCA mesophyll tissue. Leaf shape (cylindrical or semi-cylindrical) appears to have no effect on the basic anatomical structure within each type.

Typical Kranz anatomy

The leaf blade is flat, dorsiventral with amphistomatic epidermis, the adaxial and abaxial sides are clearly distinguishable. The Kranz rosettes are similar to those in grasses, though here they develop around ramified leaf veins. However the

bundle sheath is always single layered. Vast, mostly centripetally arranged chloroplasts fill the parenchymatous bundle-sheath cells (Fig. 1). The following variation had been observed among the studied species.

Amaranthoid type (Fig. 1/a)
The bundle-sheath is complete, the mesophyll is isopalisade. It is characteristic to *Amaranthus* and *Tribulus* species.

Euphorboid type (Fig. 1/b)
The bundle-sheath is complete, the radially arranged PCA tissue is palisade only on the adaxial side. Abaxial side cells are less elongated in form (overlaying a spongy mesophyll), as they adjoin the cells of the water-holding ground tissue. It is characteristic to *Euphorbia* and *Portulacca* species. In the case of *Euphorbia humifusa* scattered Kranz cells can also be seen between vascular bundles.

Atriplicoid type (CAROLIN et al., 1975, Fig. 1/c)
The Kranz cells form parenchymatous sheath around the vascular bundles, except for the gap beside the phloem, with a radial arrangement of the PCA tissue. The mesophyll by which is palisade only on the adaxial side, while its cells on the abaxial side are less elongated.

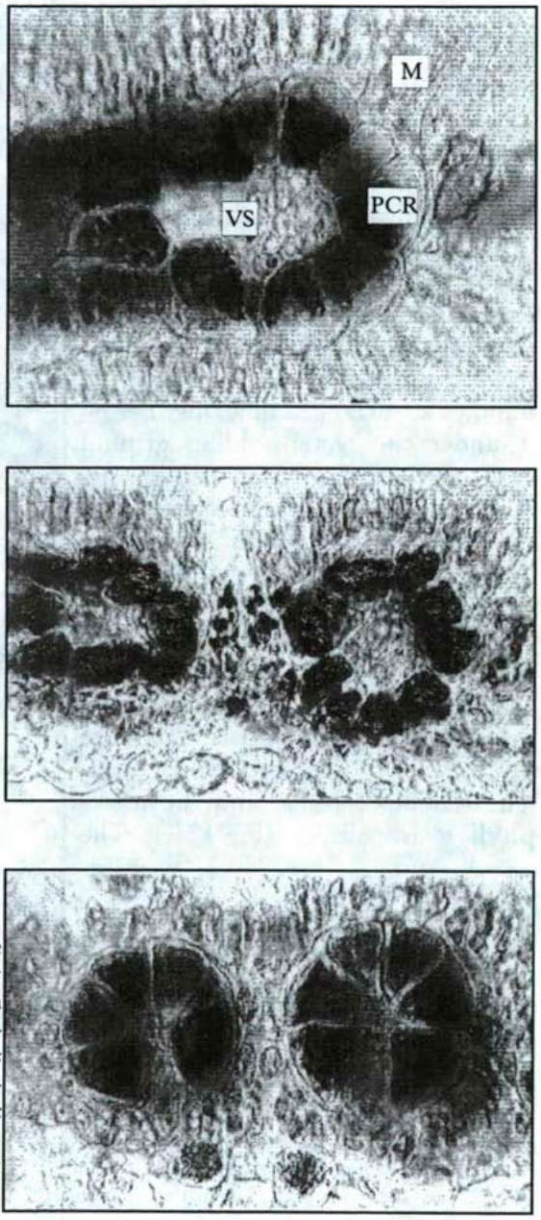

Figure 1. Micrographs of portion of leaf blade cross sections with typical Kranz anatomy (dark areas indicate the place of starch accumulation within the leaf [lugol staining]). a: Amaranthoid type — *Amaranthus retroflexus*, b: Euphorboid type — *Euphorbia humifusa*, c: Atriplicoid type — *Atriplex rosea* (x 540). Abbreviations: PCR = parenchymatous bundle sheath, VB = vascular bundle, M = mesophyll.

Non typical Kranz anatomy

Non typical types contain a „collective" Kranz sheath organised around multiple bundles in the leaf. The non-typical Kranz structures mainly occur among plants of the most extreme habitats (semidesert-like saline and sand grasslands).

Kochioid type (Fig. 2)

The leaf blade is cylindrical or flat, the epidermis is amphistomatic. The Kranz-cells (PCR tissue) form arcs along the xylem of peripheral bundles. If the leaf blade is cylindrical (*Kochia laniflora, K. prostrata*) the bundles are arranged in a ring. Below it under the water-holding ground tissue (hypodermis) the palisade parenchyma (PCA tissue) forms a continuous layer (Fig. 2/a). Mostly the leaves of this type have extensive central aqueous tissue. *Kochia scoparia* represents a special case of this type (CAROLIN et al., 1975) in which the central aqueous tissue has been suppressed and the vascular bundles opposite each other pressed together. The leaf blade is flat and the mesophyll is isopalisade (Fig. 2/c). The

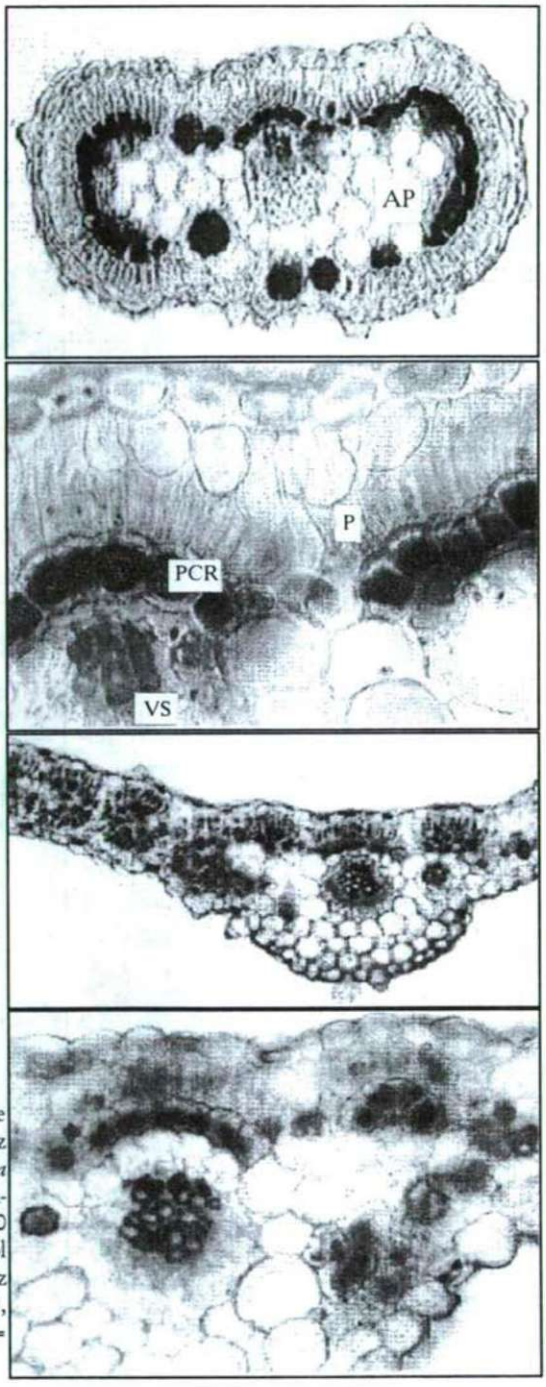

Figure 2. Micrographs of portion of leaf blade cross sections with non-typical Kranz anatomy. Kochioid type: a. and b. *Kochia laniflora* (a: x 170, b: x 540 [lugol staining]), c. and d. *Kochia scoparia* (c: x 170 [azur-hematoxylin staining], d: x 270 [lugol staining]). Abbreviations: PCR = Kranz cells (form arcs along beside the xylem), VB = vascular bundle, P = palisade, AP = aqueous tissue.

Kranz cells thus appear as a partial bundle sheath, interrupted laterally, around centric bundles towards the outer parts of the leaf transection whilst towards the centre of the leaf the aqueous tissue still separates opposite bundles and the Kranz cells appear as in other Kochioid types.

Salsoloid type (Fig. 3)

The leaf blade is cylindrical. Below the epidermis the palisade parenchyma (PCA tissue) forms a continuous layer. With this the Kranz cells (PCR tissue) connects, which also makes an unbroken sheath (Fig. 3/a and 3/b). Within this small bundles are situated in a ring. This leaf anatomy is particularly by interesting, most of the Kranz cells have no direct connection with the vascular bundles, as they adjoin the cells of the water-holding ground tissue. In the center of the cylindrical leaf blade a large vascular bundle is situated without Kranz tissue (PATRIGNANI et al., 1993). Besides the *Salsola* species this structure is characteristic to *Camphorosma annua* too (Fig. 3/c).

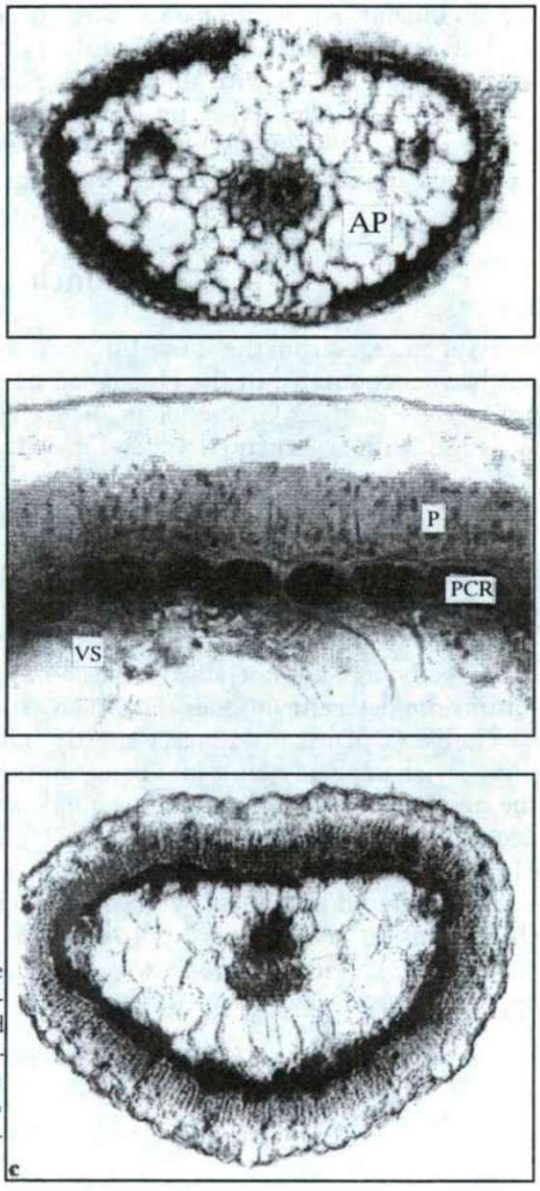

Figure 3. Micrographs of portion of leaf blade cross sections with non-typical Kranz anatomy [lugol staining]. Salsoloid type: a. and b. *Salsola kali* (a: x 50, b: x 540), c. *Camphorosma annua* (x 50). Abbreviations: PCR = „collective" Kranz sheath, VB = vascular bundle, P = palisade, AP = aqueous tissue.

In the Hungarian flora there are genuses (*Atriplex, Euphorbia*) in which either C_3 or C_4 species can be found. So far only C_3 (*Heliotropium, Sueda, Salicornia*) or C_4 (*Kochia*) species were recorded. However according to literature data (CAROLIN et al., 1975; VASZILEVSZKAJA and BUTNIK, 1981) in these genuses both C_3 and C_4 species inhabit. An additional so-called suedoid type (named after the genus *Sueda*) C_4 leaf anatomy had been distinguished in the *Chenopodiaceae* (CAROLIN et al., 1975), but no species with this structure occurs in the Hungarian flora (NYAKAS and KALAPOS, 1996). Also, no ventro-palisade structure characteristic to C_4 *Salicornia* species (VASZILEVSZKAJA and BUTNIK, 1981) was observed, as only one C_3 *Salicornia* species (*S. europaea*) grows in this country.

Conclusions

As it emerges from the above list a considerable diversity of Kranz leaf anatomy has been encountered in the Hungarian flora, since eight structural variants occur among less than 23 C_4 species in dicot. The *Chenopodiaceae* shows the greatest variation with 3 structural types for eight species, and with the most unusual Kranz anatomy variants (kochioid and salsoloid types). This high anatomical diversity also reflects the poliphyletic origin of the C_4 photosynthesis discussed in detail elsewhere (e.g. MONSON, 1989; EHLERINGER and MONSON, 1993) and the high diversity of biotopes inhabited by C_4 plants. The non-typical Kranz structures mainly occur among species of the most extreme environments (semidesert-like saline and sand grasslands). This fact shows that in forming of different photosynthetic pathways are not always the family relationships but the ecological conditions the determinant ones (KALAPOS et al., 1997).

Native C_4 plants in Hungary mostly inhabit dry grasslands or salt affected habitats, wich probably came into being during 8000–5000 years before present, when the steppe vegetation of the Great Hungarian Plain started to develop (JÁRAI-KOMLÓDI, 1987; ZÓLYOMI and FEKETE, 1994). However, noumerus C_4 species in the Hungarian flora are naturalised aliens (e.g. *Amaranthus* spp., *Euphorbia* spp.) which were introduced in the last two centuries. Human activities greatly contributed to their invasion by creating appropriate habitats for them (ruderal sites, waste ground etc.). The invasion is continuing even today, as shown by recent appearance of a new C_4 weed, *Amaranthus bouchonii* (SOLYMOSI and PRISZTER, 1984) in Hungary.

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SURFACE OF INTRAFLORAL NECTARY IN 'BESZTERCEI' PLUM CLONES

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Abstract

The intrafloral nectary of 'Besztecei' plum clones is covered with cuticle. The cuticle covering the epidermis is usually striate, but rarely it can be smooth, as well. The cuticle ridges around the stomata are often radially oriented and on the interstomatal epidermal cells they are broken at the cell borders. They may occur at smaller or greater intervals, they can be either straight or twisted. Similarly to other Prunoideae taxa, the gland epidermis consists of polygonal epidermal cells, stomata and trichomes. The outer tangencial wall of the epidermal cells is often bulging like a papilla. The stomata are able to function, and concerning their position related to the epidermal cells, they can either be at the same level, or more or less sunken, or sometimes rising above the epidermal cells. Within a single flower two ecological types may occur. The majority of the clones can be classified into the mesomorphic type. The trichomes are uni- or multicellular, short and thick or long and thin, and they prevent nectar flowing out of the flower.

Key words: plum, nectary, floral biology, epidermis

Introduction

The characteristics of the plant epidermis usually indicate the correspondance of the taxon with environmental conditions. It is known that among plant organs the flower is the most constant one, which changes the least with environmental

conditions. In the life of a plant the blooming period is relatively short, generally lasting only for a few days, while the leaf might be exposed to environmental effects for a long time. Among the parts of a flower, after the calyx, the nectary epidermis is the most similar to leaf epidermis, since according to Fahn's (1979) summary, it is covered by cuticle, broken up by stomata and at certain taxa it also has trichomes.

Studying nectary epidermis is necessary partly concerning ecotype and partly for flower biological research. Knowing the ecotype, plantation can be planned better. It is also important to know the epidermis structure from the point of view of flower- and pollination biolgy, since the gland surface may influence nectar keeping by its outstanding ribs or ridges, and consequently it has an effect on the the durability of insect attraction (Orosz-Kovács, 1990).

Fahn (1979) states about nectary epidermis that epidermal cells can be cubical or palisad-like. He classifies nectaries, and distinguishes a type that exudates nectary through stomata. Rosaceae taxa belong to this type.

The stomata are generally modified, the guard cells have usually lost the ability to close the stomatal aperture (Fahn, 1979; Gulyás, 1991), but in Rosaceae taxa the active functioning of stomata is quite common, which results in the periodicity of nectar secretion (Orosz-Kovács, 1990, 1991, 1993). The flower often becomes empty as a consequence of this rhytmicity, and the insect attraction of the flower ceases in the pauses of secretion. These phenomena underline the importance of the gland surface being smooth or broken up by ribs, which influences the length of time, while the secretory product remains in the flower.

According to Gulyás, 1991, the nectary epidermis is one layer thick, with closely attached cells of various shapes. According to him, the type of nectar secretion is determined by the structure of the epidermis.

According to Kartashova, 1965, the stucture of the nectary epidermis within the flower is the function of the position of the gland. The glandular epidermis protected by a tubular corolla is higromorphic, while flowers exposed to outer effects have mezo-or xeromorphic types. Our previous studies on Prunoideae taxa allow the conclusion (Orosz-Kovács et al., 1990; Orosz-Kovács, 1993) that the position of the stomata in the glandular epidermis is not merely the function of a gamopetalous or free corolla. Examining several cultivars of a single species, significant differences could be observed in the case of cherry (Orosz-Kovács, 1991, 1993; Orosz-Kovács and Apostol, 1993), sour cherry (Orosz-Kovács, 1990, 1991, 1993; Orosz-Kovács et al., 1993), and plum (Orosz-Kovács et al., 1990–91), although the structure of the flower was the same at each cultivar, namely the receptacle was a hypanthium sunken like a cup. It suggests that the laxial, protected surface of the hypanthium is suitable for determining the otype of the taxa.

Among other Rosaceae taxa we studied the nectary surface of apple more carefully, and we observed that the ornamentation of the cuticle differs strikingly from that of Prunoideae taxa (OROSZ-KOVÁCS et al., 1990): it can be characterised by folded laminae rather than striae.

Literature on cuticle ornamentation was summarized by METCALFE and CHALK and they also established a classification in 1979, also used by us.

About the cuticle covering nectary epidermis and its ornamentation in taxa not belonging to Rosaceae, data can be found in the works of FAHN (1979) and DURKEE (1983). Concerning the nectary epidermis of other Rosaceae species, few descriptions are known except for the studies of KARTASHOVA, 1965 and GULYÁS, 1991. In our previous works we reported on the nectary surface of Prunoideae taxa, dealing with more fruit species and several cultivars of these (OROSZ-KOVÁCS et al., 1990, 1990–1991, 1993).

Connection could be observed between nectary surface and fruit yield in the case of cherry cultivars, where cultivars with a xeromporphic nectary epidermis yielded more fruit, while those with the higromorphic type usually dropped their fruit in the slightly drier environment of Hungary (OROSZ-KOVÁCS and APOSTOL, 1993).

The cuticle ornamentation of the nectary surface in some plum cultivars was presented in one of our previous works (OROSZ-KOVÁCS et al., 1990–91), according to which the ornamentation of the cuticle is characteristic for varieties or variety-groups. Structure of primary cuticle is basically of two types: striate and reticulate. Striate form is extremely common, this is characteristic to most varieties. Between the two basic types, there are intermediate forms. Cuticular striae are radially ordinated around the stomata of the nectary epidermis covering the gland. Thin sulci acting as microcapillaries distribute the secreted nectar throughout the whole surface and retain it at the same time. Thick, striated cuticle has a good nectar retaining effect, while thin reticulum is less effective. Consequently, the former structure is more attractive for insects than the latter one. The careful examination of the nectary epidermis in Besztercei plum clones has not occured yet. Concerning the size of the nectary, however, we presented some articles previously (RÓKA and OROSZ-KOVÁCS, 1994; RÓKA et al., 1997), in which an apicultural scale of value was established based on the size of the gland. Connection between nectary structure and flower structure in plum varieties was dealt with in the work of (SURÁNYI and OROSZ-KOVÁCS, 1992). Flower morphological studies in clones of cultivated plum varieties were carried out by (SURÁNYI, 1983), who also studied the importance of the cultivar, the rootstock and the environment in plum production development (SURÁNYI, 1991), pollen viability and free of 'Besztercei' plum clones of Hungarian and foreign origin (SURÁNYI, 1996), as well as virus-sensitivity (SURÁNYI and ERDŐS, 1992).

Material and Method

The studied flowers of five 'Besztercei' plum clones originated from the basic collection of the Experimental Station of the Fruit and Ornamental Plant Production and Development Corporation in Cegléd. The living material collected for investigation was fixed in 0,2 mol glutareldehide, and washed in 0,1 mol Na-cacodilate-buffer, then dehydrated in ethylene-series. After drying on the critical point, the material was prepared for SEM-study, SEM-micrografs were executed by an SID-4 SEM adapted to a Yeol 100 C equipment. The position of stomata was studied in medial longitudinal sections of the flower. Sections were prepared by microtome after embedding in paraffine, in 5–10 μm thickness. Staining was carried out with toluidine blue.

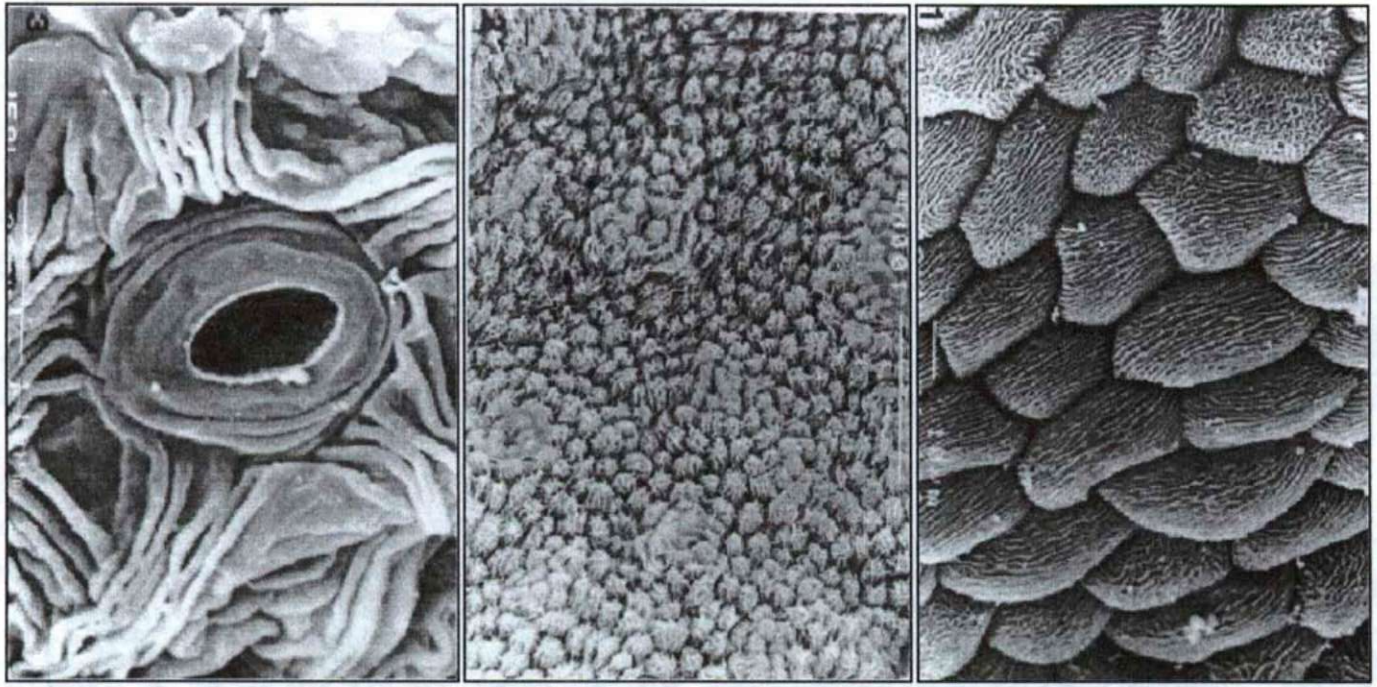
Results

Similarly to other plants and Prunoideae taxa, the nectary surface of 'Besztercei' plum clones is covered by cuticle (Orosz-Kovács et al., 1990). The surface of the nectary may change within a single variety, depending on being studied at the base or at the apex of the gland. At the Besztercei 142–59 variety the cuticular ribs of the epidermal cells at the basis of the gland are thinly scattered, evenly distributed, running parallelly, forming no ridges secondarily turned up (Fig. 1). At the apex of the gland the ribs are more frequent and their distribution is irregular. The ribs become wavy and twisted because of having turned up secondarily, and they often form ridges at the tip of the epidermal cells bulging like a papilla (Fig. 2).

The cuticle is mostly striate, mesomorphic (Fig. 8) or xeromorphic (Figs. 6, 7), but rarely it may have a smooth surface, as well (Orosz-Kovács et al., 1990), in the case of a higromorphic epidermis (Fig. 4). At the Besztercei C. 35. clone both mesomorphic and xeromorphic stomata may occur (Figs. 5, 9).

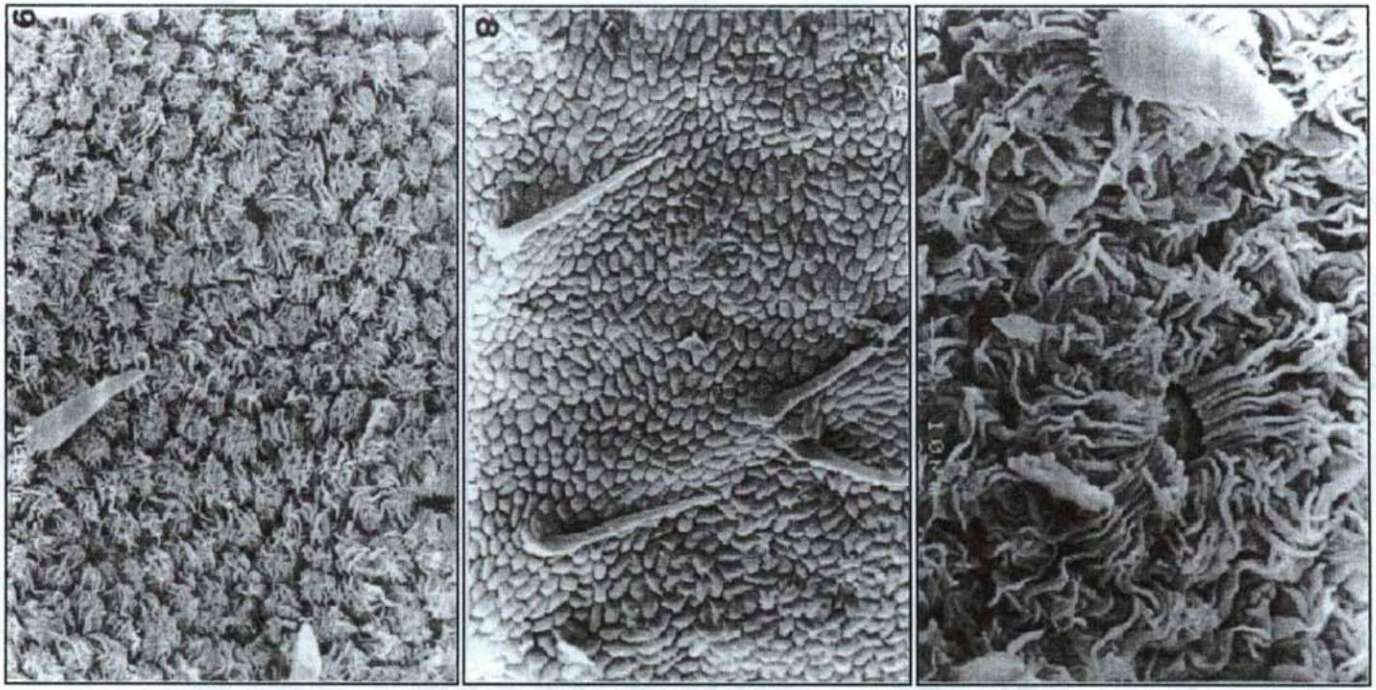
On the gland surface of the Korai (Early) Besztercei TV-46 plum clone, which has higromorphic characteristics, the epidermal cells are covered uniformly by the lamina-like cuticle, and in most cases the surface is broken only by the cell borders (Fig. 4). The stomata of the mesomorphic Besztercei 142–59 plum clone are at the same level as the epidermal cells, the ribs of the cuticle are not too frequent, but regular (Figs. 2, 3, 8). At clones with characteristically xeromorphic epidermis, the stoma is sunken below the level of epidermis cells, and also the cuticular ribs are powerful, frequent and wavy, forming irregularly folded crests on the papillae of the epidermal cells. Such is the nectary epidermis of the Besztercei C-224 plum, having xeromorphic characteristics (Fig. 6).

The cuticular ribs are often radially oriented around the stomata (Figs. 3, 5, 6, 7, 9), and the cuticular valleys, similarly to other Prunoideae taxa, act as micro-capillaries, distributing the secretory product evenly on the glandular surface, close to the stoma (Orosz-Kovács, 1990; Orosz-Kovács et al., 1990). The ribs are broken at the cell borders on the interstomatal epidermal cells (Figs. 2, 6). Ribs may occur sparsely or densely, they can be straight (Fig. 1) or twisted (Figs. 3, 5, 6, 7, 9).



Figs. 1–3: 1. Surface of the basal part of the intrafloral nectary in Besztercei 142–59 plum clone — mesomorphic type. 2. Surface of the apical part of the intrafloral nectary in Besztercei 142–59 plum clone — xeromorphic type. 3. A nectary stoma with the characteristic cuticular ribs on the surface of the apical part of the intrafloral nectary in Besztercei 142–59 plum clone — xeromorphic type.

Figs. 4–6: 4. Higromorphic nectary epidermis in Korai Besztercei TV-46 plum clone. 5. Mesomorphic nectary epidermis in Besztercei C. 35 plum clone. 6. Xeromorphic nectary epidermis with sunken stomata in Besztercei C. 224.



Figs. 7–9: 7. Short, thick, pointless trichomes on the xeromorphic nectary epidermis in Besztercei KD-10 plum clone. 8. Long, thin, tapering trichomes of the floral nectary epidermis in Besztercei 142–59 plum clone. 9. Short, thick and tapering trichomes of the intrafloral nectary of Besztercei C. 35 plum clone.

Around the stomata the cuticle often forms wrinkles in the shape of concentric circles (Figs. 3, 5). The nectar gap is mostly oval after the cuticle has been torn (Fig. 3).

At the Besztercei plum clones trichomes often occur on the nectary surface. These trichomes are coverhairs, they can be unior multicellular, short and thick, as e.g. at the Besztercei KD. 10 or C. 35. clones, or long and tapering, as in the case of the 142–59 clone (Fig. 8). The trichomes prevent nectar flowing out of the flower.

The results make clear that the intrafloral nectary surface at the clones of the Besztercei plum cultivar group is highly variable, although it concerns an intraspecific taxon. Cuticle ornamentation and the position of stomata in relation to the epidermal cells indicate primarily the ecotype, but the characteristics of each clone can be observed, too. For identification of a broad scale of clones, the study of further clones can be suggested.

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DISTRIBUTION OF PLANT NUTRIENTS IN THE SEEDS AND SHOOTS OF *CHENOPODIUM RUBRUM* L. VAR. *PUSILLUM* HAUSSKN. ALONG AN ENVIRONMENTAL GRADIENT

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Abstract

Distribution of plant nutrients (potassium, nitrogen and phosphorus) was studied in shoots and seeds of *Chenopodium rubrum* var. *pusillum* along a transect which was marked out on the bottom of a flood-plain lake of Körös river. The sampling of plants was performed on seven occasions paralel with the drying out of the lake. The plants in a transect point were at different stages of their development. The nutrient condition of the soil was also measured.

During the vegetation period the potassium and the nitrogen contents of shoots showed decreasing tendencies, the phosphorus content of shoots increased. The potassium content of seed decreased, the nitrogen and the phosphorus content was shown to be constant in a sample site.

Relationships between edaphic factors and the nutrient content of plants might be informative in cases where the contents of nutrient are close to constant during the period of investigation. If the nutrient contents of plants show tendentious changes, a single occasional transect study may (but not necessarily) give questionable results. The mechanical application of tests suggests that there are no strong correlations between phosphorus content of shoots and the soil. In this study only close correlation of phosphorus content of shoots and seeds can be rendered possible, in spite of that fact the mechanical application of correlation tests apparently indicates significant correlation between the potassium and nitrogen content of the shoots, and the soil.

Key words: mud vegetation, nitrate, nitrogen, nutrient uptake, phosphorus, potassium, resource allocation, seasonal dynamics.

Introduction

Relationships between the nutrient content of plants and the soil have important ecological significance (GERLOFF, 1976; GRIME at al., 1988). The studies in this subject are built on the supposition that the resources of the soil distribute among the competitive plants according to their ability of nutrient uptake and utilization, and that these competitive relations can be followed by the element analysis of plants (organs, tissues) (GRIME, 1977; CHAPIN, 1980; ERNST, 1983; LEE et al., 1983; MARRS et al., 1983; AUSTIN et al., 1985, TILMAN, 1985; 1986; KOVÁCS et al., 1995a). As the determination of starting parameters of an investigation needs exact experimental conditions and design, most of the studies were performed in phytotron under controlled conditions (c. f. TILMAN and WEDIN, 1991). Field studies to investigate resource allocation and competition are performed in rare instances (TILMAN, 1984; WILSON and TILMAN, 1991; KOVÁCS et al., 1995b). An important part of these field studies is devoted to the investion of the distribution of nutrients in a plant during the vegetation period (CHAPIN and KEDROWSKI, 1983; GEBAUER et al., 1984; NADELHOFFER and ABER, 1984). Sometimes the distribution of plant nutrients in an environmental (nutrient) gradient is the subject of study (PARRISH and BAZZAZ, 1982; PASTOR et al., 1984). This study belongs to the latter type, but sequential sampling of plant material was performed to detect relationships existing between the nutrients of the soil and the plants, as well as the changes of nutrient content during plant development.

As the interpretation of field data would have required too much consideration in a complicated vegetational situation, a very simple subject has been choosen: *Chenopodium rubrum* var. *pusillum* colonizes almost bare soil in lakes of flood plains after the water recedes. The total cover of vegetation is low, therefore the effects of interspecific competition can be omitted from consideration. This species forms monodominant stands in zones where its coverage is high (c. f. Fig. 4/B). *Chenopodium rubrum* var. *pusillum* is a typical mud-plant, which characterizes the *Nanocyperion* KOCH ex LIBBERT 1932 or *Heleochloo-Cyperion* (BR. -BL. 1952) PIETSCH 1961 alliance of mud vegetation (PIETSCH, 1973a,b; PIETSCH and MÜLLER-STOLL, 1974). Its shoots are 3–7 cm in tall. Its special adaptation to the short available vegetation period is „the fast life cycle", the early flowering and the fast ripenings of fruits and seeds (c. f. ERNST, 1983). The nutrients are in high availability in the soil, therefore none of them is a limiting factor in growth.

Materials and methods

Study area

The studies were performed along the Körös river, at the bottom of a flood-plain lake located at a distance of 200 m from the river near Békésszentandrás village (Fig. 1/A). Paralel with the continuous evaporation of water, the terrestrial vegetation forms well distinguished zones on the bed soil. On the part of the lake that is under water coverage for the longest period, vegetation of higher plants does

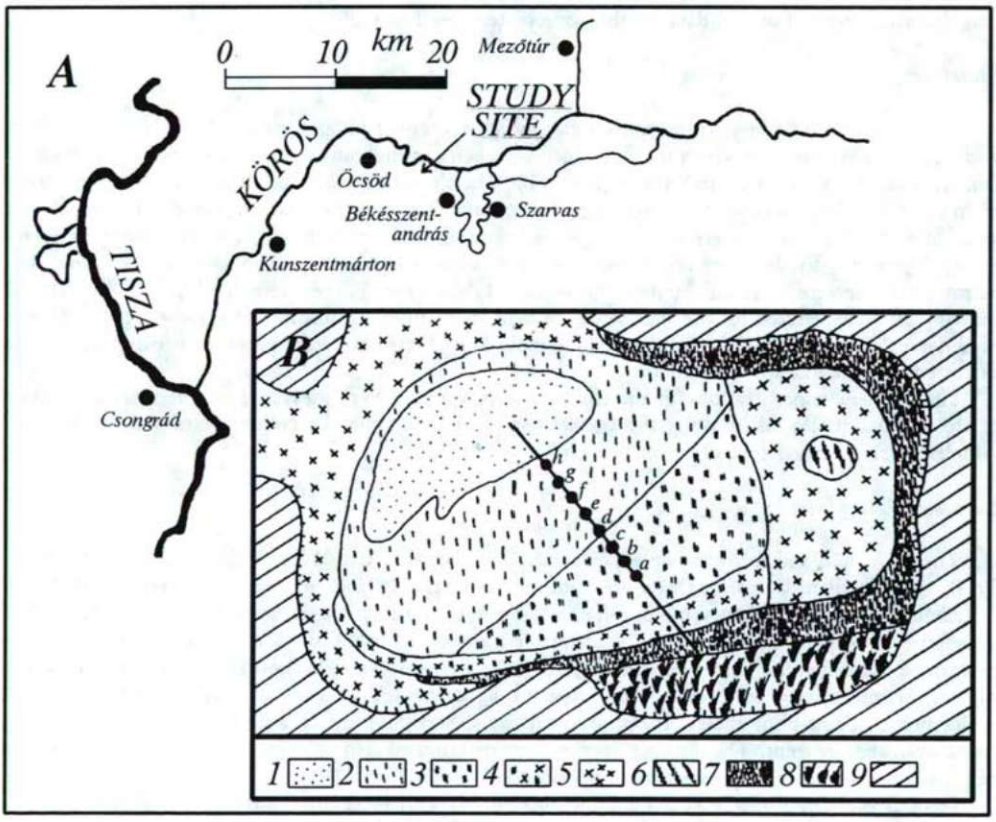

Figure 1 Geographical location of the study area (A). Vegetation of the lake and the location of sample sites (B); 1: *Botrydietum granulati* HÜBSCHMANN 1957 x *Riccio-Physcomitrelletum* (ALLORGE 1921) HÜBSCHMANN 1957 komplex, 2: *Dichostylido-Heleochloëtum alopecuroidis* (TIMÁR 1950) PIETSCH 1973 *chenopodiosum rubri*, 3: *Dich.-Hel. gnaphaliosum uliginosi*, 4: *Dich.-Gnaph. gnaph.* x *typ.* transition, 5: *Dich.-Hel. typ.* x *Eleochareto aciculari-Schoenoplectetum supini* SOÓ et UBRIZSY in UBRIZSY 1948 *eleochariosum* komplex, 6: *Bolboschoenus maritimus* fac., 7: *Agrostis stolonifera* fac., 8: *Caricetum gracilis* ALMQUIST 1929, 9: *Salicion triandrae* and *Salicion albae* TH. MÜLL. et GÖRS 1958. (Nomenclature follows BORHIDI 1996, HÜBSCHMANN 1957.); a — h: sample sites, distance between 'a' and 'h', is about 20 m.

not occur. Only algae (e. g. *Botrydium granulatum*), liverwort (*Riccia cavernosa* HOFFM. em. RADDI) and mosses (e. g. *Physcomitrium pyriforme*) can be found on the ground (RUNGE, 1960; ANT and DIEKJOBST, 1967). The next zone towards the shore is dominated by *Chenopodium rubrum* var. *pusillum*, and the next covered by the annual *Gnaphalium uliginosum* and *Heleochloa alopecuroides*. The transition between the two zones is close to continuous. The dominant species of the further zone is *Agrostis stolonifera*, which forms a dense carpet on the soil. It partly consists of specimens transformed into terrestrial (from aquatic) forms, but mainly consists of the new seedlings of the species. The first zone, which is covered by permanent perennials, is dominated by *Carex gracilis* and other *Magnocaricion* species (Fig. 1/B). For a more detailed cenological description see BAGI (1984; 1991). Due to the shallow water and the slow decrease of the water level, wide zones of different vegetation distributions developed. The detailed investigations were restricted to the *Chenopodium rubrum* dominated, and neighbouring zones. For soil data on the zone system see BAGI (1988).

Sampling

The zone where *Chenopodium rubrum* var. *pusillum* occured in appropriate cover, was about 20 m wide. Along a transect, in which the difference between the highest and the lowest part was about 15 cm, eight sample sites were marked out (Fig. 1/B). The shoots of *Chenopodium rubrum* were collected from these sites in paralel with the receding of the water. The collection was repeated about every two week in each sample site, where shoots could be found in sufficient numbers, and in appropriate stages of development. Samples were taken seven times at the higher relief, but only once at the lower. The plants were harvested starting 31 July and ending 1 Nov. 1986.) The most developed shoots (15–20 individuals) were selected for the analyses, each time from different quadrats of a transect point. Shoots of different ages were collected from each sample site. After the fruits become mature, seeds were extracted from the collected shoots.

The nutrient concentrations of the soil in a sample point were measured on the basis of analysis of three soil samples taken along the transect at the time of the last collection of shoots. The soil samples were collected to a depth of five cm.

Laboratory procedures

The plant and soil analyses were performed in accordace with Hungarian standards (cf. BUZÁS 1988). Additional methods, or those not conforming to these standards, are referenced separately.

Plant analyses: The plant samples (after rinsing with distilled water) were dried out at 60°C. The liquefaction of plant samples for the determination of phosphorus and potassium content was performed with a nitric acid : perchloric acid (5:1 v/v) mixture. The liquefaction to determine the nitrogen content was performed using Kjedahl apparatus. The determination of potassium was performed by flame photometry, the phosphorus by spectrophotometer after reaction with molybdate — metavanadate reagent. The nitrogen content was determined also colorimetric way after a procedure of phenol — sodium nitroprusside reaction (Felföldy-method).

Soil analyses: The available potassium and phosphorus were determined after extraction with calcium lactate, the nitrate-nitrogen of the soil extracted with distilled water (1:5 soil : water w/v ratio). The determination of potassium was performed by flame photometry, and phosphorus by spectrophotometer after reaction with sodium molybdate — ascorbic acid reagent. The nitrate concentration of the extract was determined by the phenol disulfonic acid method (BALLENEGGER, 1953), and also using colorimetric method.

The determinations were run in triplicate.

Statistical analysis

Statistical evaluation of the data was restricted to the Spearman rank order correlation analysis. The low number of data pairs made necessary the application of nonparametric statistics, therefore tests of normal distribution were omitted.

Results

Distribution of plant nutrients in shoots and seeds

The nutrient contents of shoots and seeds are close to similar in value, only the potassium content is considerably higher in the shoots than in the seeds. The concentration of a nutrient varies between wide limits in the shoots: For example, the changes in phosphorus content in some sites were more than 200 % during the period of investigation. At the same time, the phosphorus and nitrogen content of the seeds seems to be constant at a sample site (transect point), but between the sites there may be important differences in nutrient contents (Fig. 2).

At a given transect point, the potassium content of shoots shows a close to linear decrease in the early stages of plant development, but it becomes constant in older plants (Fig. 2/A). The decrease in potassium content of the seeds seems to be exponential. This decrease is especially fast at the transect points of lower reliefs (Fig. 2/B). The nitrogen content of the shoots at each given transect point, decreases linearly over the period of sampling (Fig. 2/C). The nitrogen content of the seeds, over time, is close to constant at a transect point, only a very slight decrease can be detected (Fig. 2/D) (c. f. KIRKBY, 1981). A linear increase can be observed in the phosphorus content of shoots (Fig. 2/E). The phosphorus content of the seeds seems to be constant (Fig. 2/F).

Along the transect, the distribution of nutrient contents of plant samples collected at the same time can be followed in Fig. 3. The potassium content of shoots seems to be constant in the outer (higher) parts of the transect, but steep increases can be detected in the lower parts of the lake. A similar distribution in the potassium content of seeds can be observed (Fig. 3/A,B). The linear increase in the nitrogen content of shoots can be measured towards the deeper parts of the lake along the transect (Fig. 3/C). The phosphorus content of the shoots is close to constant in the outer parts, but a steep decrease can be observed in the inner ones (Fig. 3/E). The nitrogen and potassium content of the seeds show more complicated distribution along the transect: The phosphorus content shows a clearly recognizable maximum at the transect point 'd' (Fig. 3/D,F).

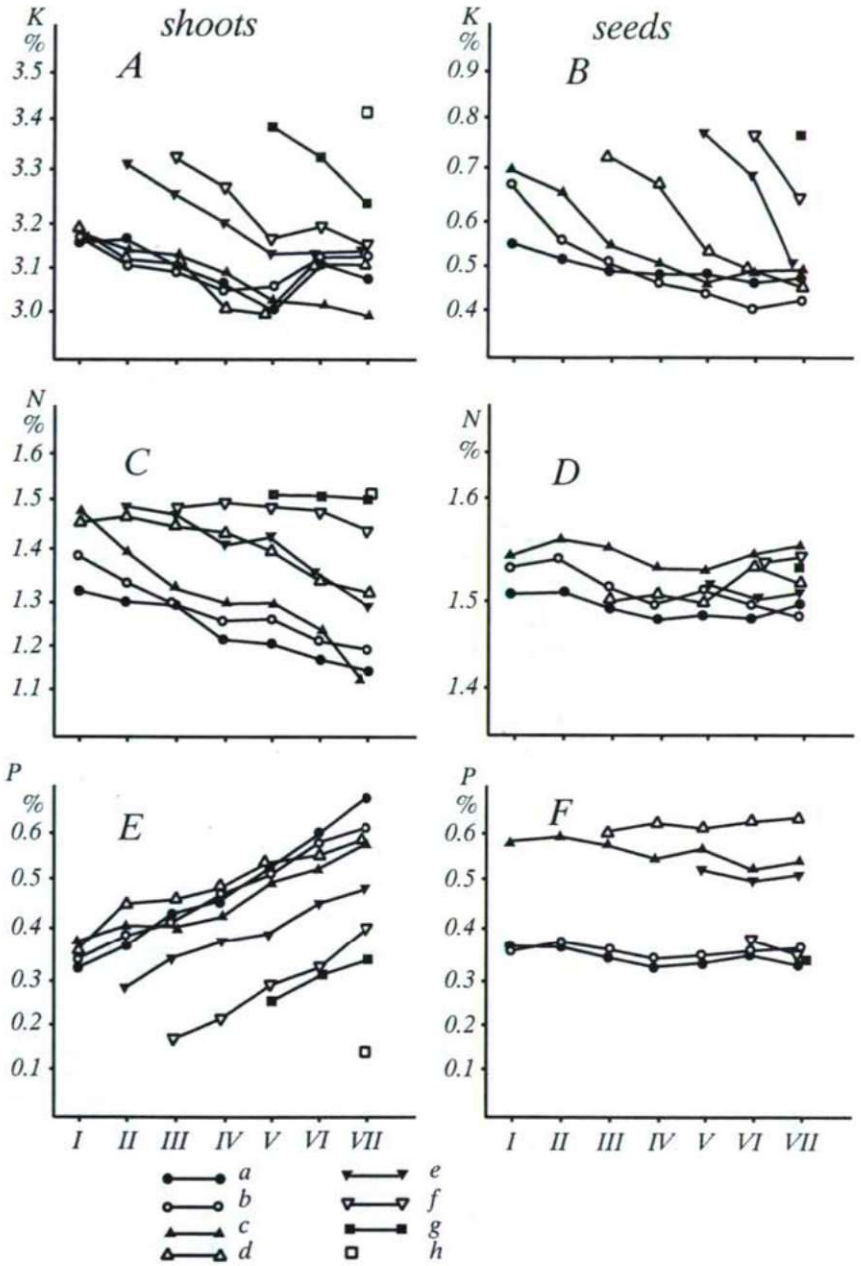

Figure 2 Changes of nutrient content in the transect points in relation to vegetation period. Dates of samplings are I–VII in order 31. 07, 12. 08, 30. 08, 12. 09, 03. 10, 15. 10 and 01. 11. The transect points (a–h) are connected with the lines.

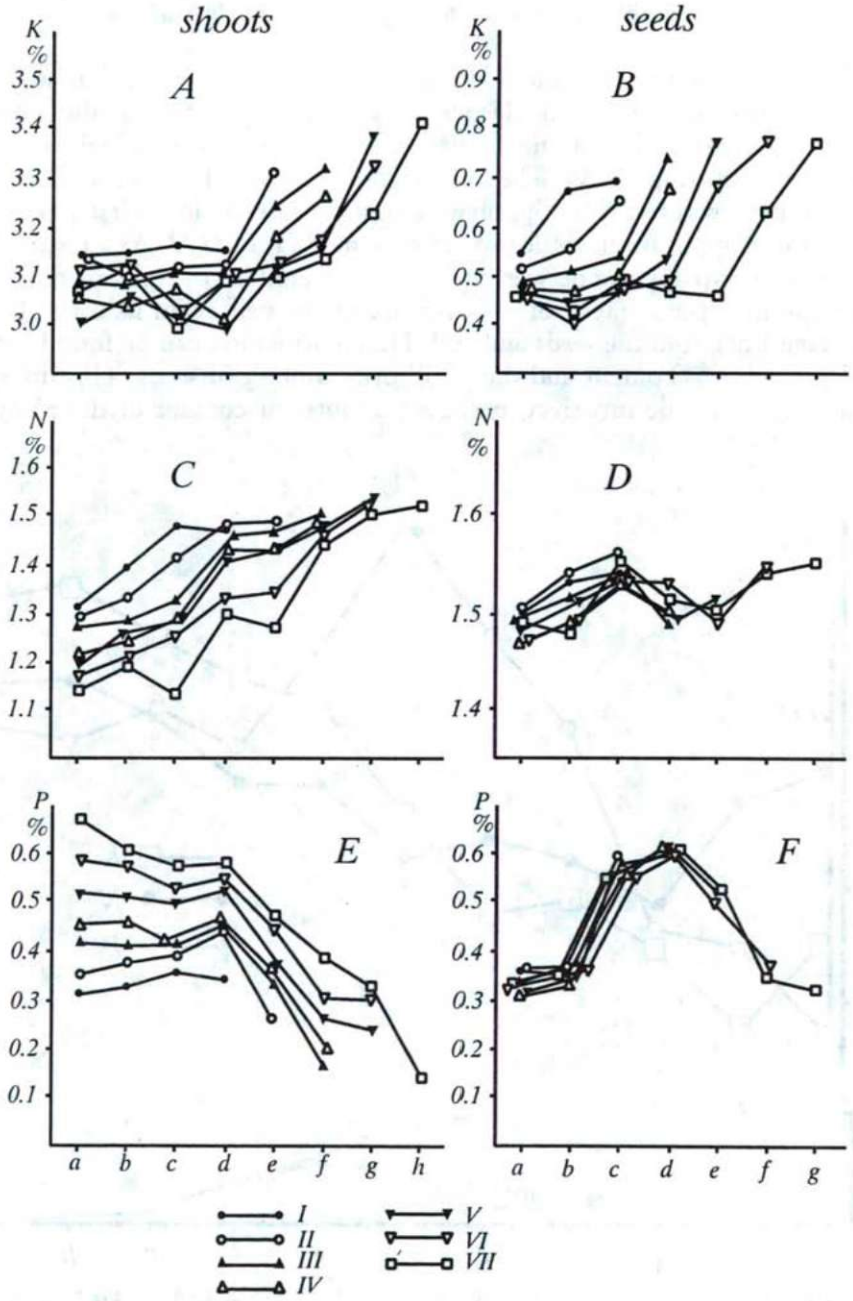

Figure 3 Distribution of plant nutrients in plant shoots and seeds along transect 'a–h'. The lines connect values that were sampled at the same time (I–VII). See Fig. 2.

Connection between plant nutrient content of plants and the soil

The concentration of available potassium and nitrate nitrogen in the soil shows tendency to increase towards the deeper parts of the lake. The available phosphorus has a maximum value at the transect point 'd', but is also high at transect points 'c' and 'e' (Fig. 4/A). The relationship between the soil and the plant nutrient content was tested by Sperman rank order correlation. Firstly the results of a mechanical application of the test are presented (Tab. 1/A). As a result of this, a close relationship appears betwen the potassium content of soil and shoots, and between the nitrogen content of soil and shoots, as well as (in its early developmental stages) between the seeds and soil. High correlation can be found between the soil phosphorus content and the phoshorus content of seeds. This interpretation, however, may be incorrect, because the nutrient content of the plants had

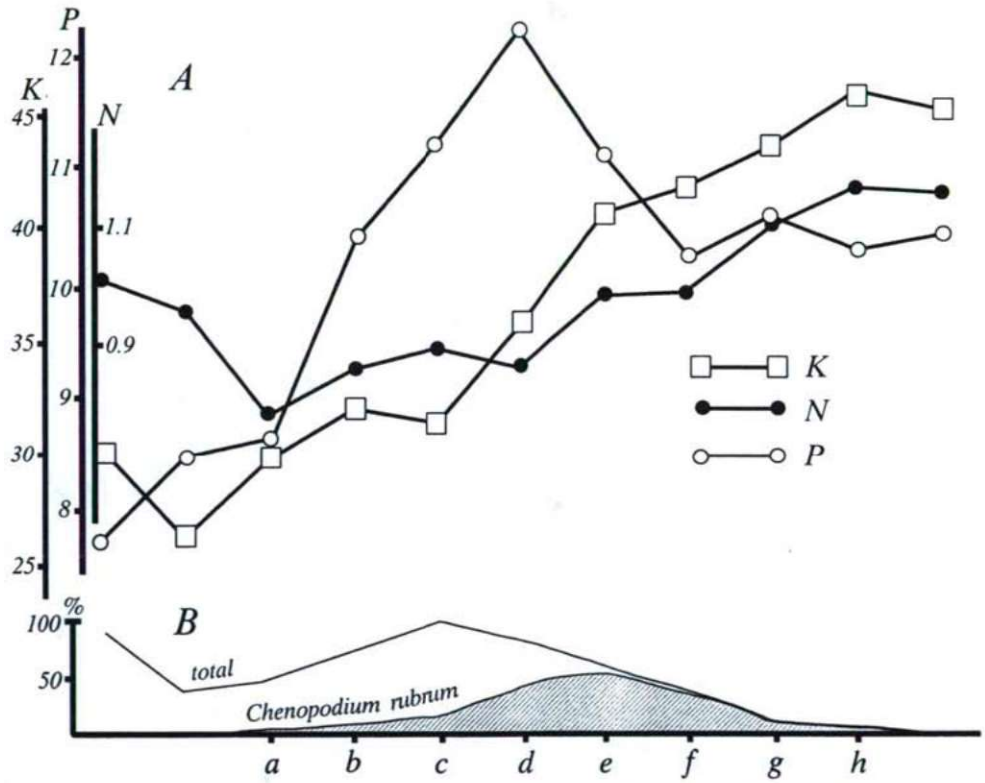

Figure 4 Distribution of plant nutrients in the soil along the 'a-h' transect (A), K: mg K_2O/100 g soil, N: mg NO_3/100 g soil, P: mg P_2O_5/100 g soil. Coverage of vegetation and *Chenopodium rubrum* var. *pusillum* along the transect (B).

changed during the plant development in several cases (see above). The interpretation would be more correct if plant material of similar developmental stages was compared. A possible resolution of this problem is to compare plants that are close to similar distances from the receding water. A graphical illustration of the selection (and some cases of the reduction) of such a sample can be followed in Fig. 5. The results are derived from the comparison of the corrected data of plant materials and the soil conditions, and can be studied in Tab. 1/B. (Tab. 1/B). The most important difference is that the close correlation between the potassium concentration of soil and the potassium content of shoots, as well as between the soil nitrogen and nitrogen content of shoots, are lost, but high correlation arises between the phosphorus content of the soil and the shoots. The distribution of phosphorus content in the soil and in the seeds remains in close correlation.

Table 1. Connection (Spearman R) between nutrient content of plants and the soil. ***: P<0. 01, **: P<0. 05, *: P<0. 1, age in weeks.

(A) date	n K shoots	N shoots	P shoots	n K seeds	N seeds	P seeds
31.07	4 0.400	0.949*	0.800	3 0.500	1.000***	0.500
12.08	5 0.100	0.821*	0.700	3 0.500	1.000***	0.500
30.08	6 0.783*	0.794*	0.257	4 0.800	0.632	1.000***
12.09	6 0.486*	0.706	0.290	4 0.400	0.949*	1.000***
03.10	7 0.893***	0.927***	-0.036	5 0.600	0.616	1.000***
15.10	7 0.883***	0.927***	-0.179	6 0.829*	0.441	0.943***
01.11	8 0.905***	0.771**	0.000	7 0.429	0.661	0.786**
(B) age						
2–3	5 0.900**	0.872*	0.900**			
4–5	5 0.500	0.154	1.000***			
6–7	5 -0.500	-0.316	0.800	5 0.600	0.359	0.900**
8–9	5 -0.300	-0.667	0.900**	5 0.600	0.474	1.000***
10–11	4 -0.200	-0.632	1.000***	5 -0.800	0.205	0.900**
12–13	3 0.500	-0.500	1.000***	4 0.200	0.949*	1.000***

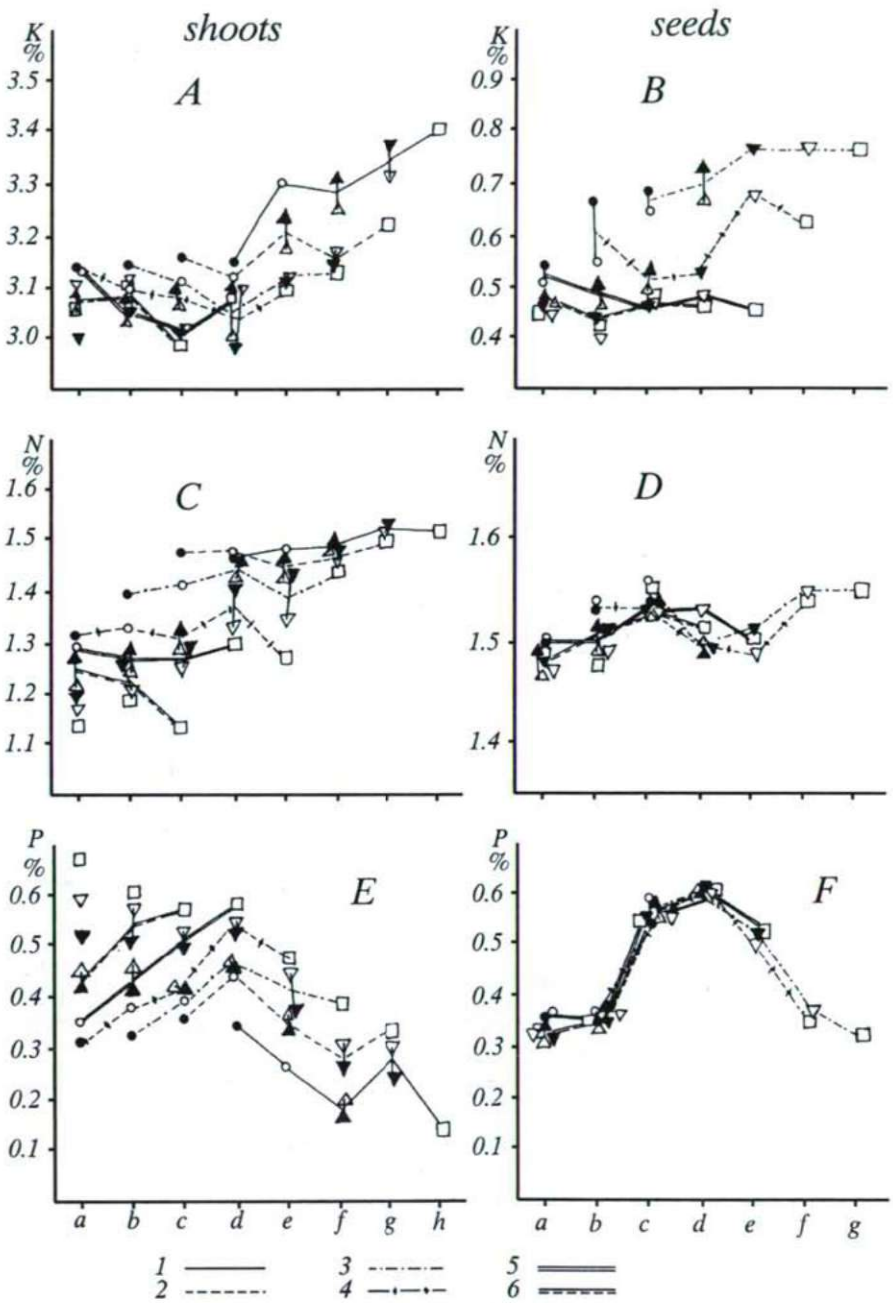

Figure 5 Nutrient content of plant materials. The lines connect nutrient data of plants that are (presumably) close to similar stages (1–6) of development (c. f. Fig. 3, and Tab. 1/B)

Discussions and Conclusions

Relationships between edaphic factors and the nutrient content of plants may be informative in cases where the concentrations are close to constant during the period of investigation. If the nutrient contents of plants show tendentious changes, a single transect study may give questionable results: For example, as a result of this study, it is probable that false significant correlations arose between the potassium as well as the nitrogen content of shoots and the soil. The mechanical application of tests suggests that there are no strong correlations, in, for example, those which exists between the phosphorus content of shoots and the soil. If the nutrient contents of plants show tendentious changes, a single, occasional transect study may (although not necessarily) give questionable results. In this study the phosphorus and potassium content of seeds are close to constant. In spite of this, only the phosphorus content of the seeds shows probable significant positive correlation to that of the soil. The relationship between the seed nitrogen content and the soil nitrate concentration is dubious. At the same time, the tendentiously decreased phosphorus content of the shoots is probably in close correlation to the available phosphorus in the soil.

In spite of the intentionally choosen elementary field samples (c. f. PEMADASA and LOWELL, 1974; CHAPIN, 1980; GOLDBERG and MILLER, 1980; WOODMANSEE and DUNCAN, 1980; ERNST, 1983; LEVINE et al., 1998), interpretation of correlations between the distribution of plant nutrients in the plants and the soil needs due foresight. In the case of dynamic or more complex vegetation situations, since the interpretation of field data requires too much consideration, the ecological investigation of relationships between the plant nutrient conditions and the edaphic factors (not to mention the competitive relations) needs controlled conditions particularly in relation of nutrition (SHAVER and MELILLO, 1984). If the production of controlled conditions is difficult (e. g. in forest sites), the investigation of the seasonal nutrient cycle is of the utmost importance (COLE, 1981; JAKUCS et al., 1981; VITOUSEK, 1982; BIRK and VITOUSEK, 1986).

Acknowledgement

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SOIL FAUNA STUDIES IN A BEECH FOREST I. COMPARATIVE STUDY IN FOREST, FOREST MARGIN AND CLEAR-CUT AREA IN HUNGARY

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Abstract

The soil fauna of a beech forest and the adjoining clear-cut area was studied during a four-year period in the Bükk Mountain (Northern Hungary). Samples were taken each month from April to November by pitfall traps. 17 taxa of the soil fauna were examined *(Scarabaeidae, Carabidae, Staphylinidae, Coleoptera, Heteroptera, Formicidae, Isopoda, Araneidae, Phalangidae, Pseudoscorpionidae, Julidae, Glomeridae, Polydesmidae, Lithobiidae, Geophilidae, Scendylidae,* and *Blattidae).* We have demonstrated that the fauna of the forest, forest margin and clear-cut area can be separated by multivariate methods. From zoological point of view, the width of the forest margin was approximately 10 metre. Our result also suggest that the distance from the forest appears to be more important in determining the faunistical composition of the sites than the exposure on the slope. *Collembola* and *Oribatidae* were the dominant taxa of the forest. Diversity of predator species was lower, while the abundance of litter-decomposers were higher in the forest than elsewhere. The abundance of *Coleoptera* was also higher in the forest than in the clear-cut area. The fauna of the forest margin was more similar to the fauna of the clear-cut area than to the fauna of the forest.

Key words: beech forest, clear-cut area, forest margin, soil fauna.

Introduction

This article is related to the „Rejtek Project" Research Programme, which involves a study of the processes of secondary succession after clear-cutting of a beech forest developing on shallow soil derived from limestone in the Northern Hungarian Central Range (JAKUCS, 1987).

A comparative faunistical study was carried out on the sampling sites in the research area, to examine the changes in the meso- and macro-fauna of the soil after clear-cutting. There were studied 16 broad taxa of the soil fauna *(Scarabaeidea, Carabidae, Staphylinidae, Coleoptera, Heteroptera, Formicidae, Isopoda, Araneidae, Phalangidae, Pseudoscorpionidae, Julidae, Glomeridae, Polydesmidae, Lithobiidae, Geophilidae, Scendylidae* and *Blattidae)* to explore whether there were any differences between the soil fauna of the forest, forest margin and clear-cut area. These taxa represent different strategies of resource utilisation, reproduction, etc., i. e. to some degree different guilds of the fauna. We were also interested in the problem of habitat preference of broader taxa, not species.

Sampling area, sampling methods and data processing

The research area is situated in the Northern Hungarian Central Range (Bükk Mountains) on a broken karst limestone area in the eastern part of the southern Bükk. The area was marked out in 1980 on a wider, south-narrowing ridge covered by an approximately 100–200 year old beech forest *(Melico-Fagetum)* in 1980. The closure of the canopy ranged from 75% to 80%. Shrubs developed considerably only in the forest covering the south-western slope. In January of 1981 clear-cutting was performed on an area of 4 hectares for experiments. A more detailed description of the studied area and the research programme are in the paper of JAKUCS (1987).

The soil fauna was studied in samples of size 25x25 cm, which is a standard plot size (LOKSA, 1966; MÜLLER, 1965). Sieving was used to separate animals from the soil and vegetable material. The remaining organisms were picked up individually from the soil samples. Pitfall traps were used to study the macro-fauna; it is applied extensively to study surface dwellers, such as spiders, *Collembola,* ants and *Coleoptera* beetles. Glass containers (7.5 cm in diameter) was used as a trap, with ethylene glycol as killing-preservative. Traps were located in three rows on the north-eastern slope. The first row of traps was situated on the upper part, the second on the middle part, and the third at the foot of the slope. The first two traps in the row were in the forest, the third in the forest margin, the fourth–sixth in the clear-cut area, and the seventh in the forest margin on the opposite side of the clear-cut area. Samples were taken each month from April to November.

The fauna of sample localities were analysed by multivariate methods. These techniques allow the comparison of a series of samples whose variation is considered to be due to several causes. Cluster analysis and principal coordinates analyses were performed via the Euclidean distances with oblique coordinates (ORLÓCI, 1978). The total linkage method was used to cluster the traps. Calculations were done by the NuCoSA package (TÓTHMÉRÉSZ, 1993).

Results and discussion

Both cluster analysis and principal coordinates analysis suggest that there are three typical parts of the study area based on the distribution of meso- and macro-fauna: forest, forest margin and clear-cut area. Three groups of sample sites are demonstrated by the dendrogram of the cluster analysis (Fig. 1). The first one (clear-cut area) comprises traps 1, 8, 16, 19, 20, 21 and 22; the second one (forest

margin) includes the traps 2, 3, 9, 12, 13, 10 and 11; and the third group (forest) includes the traps 4, 5, 6, 7, 14, 15, 17, and 18. The three groups can be clearly observed.

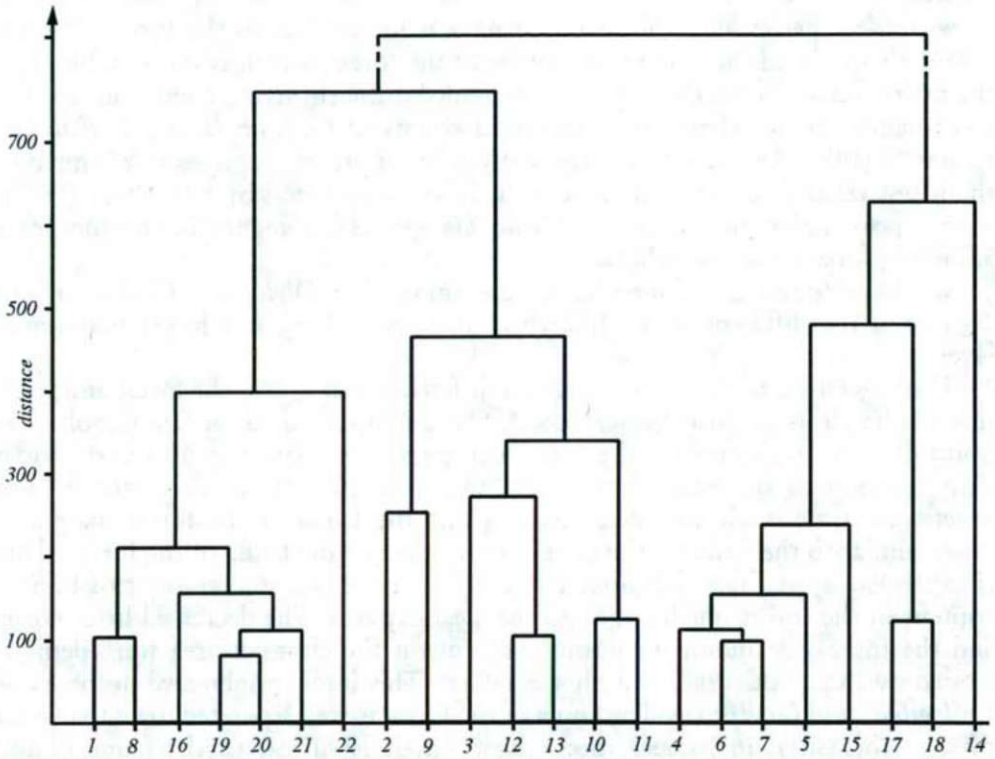

Figure 1 Dendrogram of the sampling sites basen on faunistical composition. The Euclidean distance with oblique coordinates, and the total linkage method was used.

The forest soil is covered by thick litter, and there are only a limited number of annuals. Therefore, in the forest the litter-decomposers, and species living in the litter are predominate. Diversity of predator species is limited in the forest compared to the other sites. This was also also demonstrated by KLEINERT (1977) and CYKOWSKY (1975). *Collembola* and *Oribatidae* are the dominant taxa of the soil fauna of the forest, and at the same time the litter decomposers of the soil fauna are also represented here in a greater proportion than in the clear-cut area. The number of *Coleoptera* individuals, which are faeces-decomposers, is significantly higher in the forest than in the clear-cut area. The soil microarthropods were studied by SEASTEDT and CROSSLEY (1981), and also by ABBOTT and CROSSLEY (1983). They provided evidence that the diminishing litter cover and the

increasing amount of humus resulted in the increasing numbers of *Collembola* and *Oribatidae* similarly to our result.

The soilfauna of an old oak forest and of a clear-cut *Querco-Fagetum* forest were studied by ŠUSTEK (1984), who found that the abundance of *Carabidae* and *Staphyinidae* species after the clear-cutting was lower than in the forest. ŠUSTEK (1984) also claimed that the microclimate of the forest, which is more stable than the microclimate of the clear-cut area, provided sufficient living conditions for the less tolerant species. This led to increased counts of *Collembola* and *Oribatidae*. KABACIK (1957) demonstrated that the diversity of predator species was limited in the forest relative to the clear-cut area. This was also found by KLEINERT (1977), who reported that the counts of *Carabidae* species are higher in the meadows adjoining forests than elsewhere.

We have found no differences in the amount of *Diplopoda, Chilopoda* and *Isopoda* in the different sites. Differences may be realised at a lower taxonomic level.

The vegetation of the forest margin is different from that of the forest and from that of the clear-cut area (MÉSZÁROS, 1988). Based on our data, from zoological point of view, we estimated the forest margin as approximately 10 metres wide. This agrees with the estimation of MÉSZÁROS (1989), which was based on the vegetation. Our result also demonstrates that the fauna of the forest margin is more similar to the fauna of the clear-cut area than to the fauna of the forest. This is explained by the fact that litter cover is much less, and the amount of humus content of the soil is much larger on the clear-cut area. The decreased litter cover and the increased amount of humus content on the clear-cut area were demonstrated by BODNÁR (1987) and HOLES (1989). This leads to increased numbers of *Collembola* and *Oribatidae*. The fauna of the forest is well separated from the fauna of the other sites. In some respects, however, it is similar to the fauna of the northern forest margin. The similarity of the forest and the northern forest margin is caused by the microclimatic similarity of these sites; the humidity is high, the soil temperature is low, and the insolation is lower in the northern forest margin than in the clear-cut area.

On the clear-cut area, the meso- and macro-fauna of the soil are relatively homogeneous, i. e. the similarity of the fauna in the traps was relatively high. It is also noteworthy that the distance from the forest appears to be more important in determining the similarities of the fauna of the sites than the exposure on the slope. The microclimatic conditions (soil temperature, humidity, evaporation, etc.) are extreme on the clear-cut area. Thus, only those species with a broad ecological tolerance are not influenced strongly by these factors. The diversity and phytomass of the vegetation increased after the clear-cutting (KATONA and TÓTHMÉRÉSZ, 1985); this resulted in increased numbers of phytophages and predators among the soil fauna.

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EFFECTS OF CARBODIIMIDES COUPLING AGENTS ON THE PROPERTIES OF IMMOBILIZED GLUCOAMYLASES

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Abstract

Glucoamylase (1,4-α-D-glucan glucohydrolase, EC 3. 2. 1. 3) produced by *Aspergillus niger* was immobilized on polyacrylamide beads possessing carboxylic functional groups activated by water-soluble carbodiimides bearing different substituents. The most favourable carbodiimide was N-*t*-butyl-N′-dimethyl-aminopropyl carbodiimide methyl iodide. The structure of the carbodiimide was found to influence the pH and temperature dependence of the catalytic activity, and the substrate specificity of the immobilized enzymes.

Key words: glucoamylase, carbodiimides, immobilized

Introduction

Carbodiimides are excellent reagents for the activation of carboxylic groups of supports under mild conditions (WELIKY and WEETALL, 1965; MOSBACH, 1970; MOSBACH and MATTIASSON, 1970). For successful immobilization, the amino acid side-chains essential for the catalytic activity should mostly remain intact during the immobilization. Therefore the reactive sites of the activated support should react with the amino acid side-chains on the enzyme surface, rather than with those of the active site. In the case of an activated support bearing bulky reactive groups of the O-acyl-isourea type, access to the active site hole is sterically hindered

and, consequently, the undesirable reactions become inhibited. On the basis of the structural characteristics of an enzyme to be immobilized, the most adequate carbodiimide can be selected for activation of the support (SZAJÁNI et al., 1991). In the present work, the effects of disubstituted carbodiimides have been studied using glucoamylase, an enzyme of great practical importance in the starch processing.

Materials and methods

Materials: Glucoamylase (1,4-α-D-glucan glucohydrolase, EC 3.2.1.3) with a specific activity of 900–1500 units/g protein was produced by *Aspergillus niger*. One unit is defined as the amount of enzyme that catalysed the liberation of 1 g of D-glucose from soluble starch per hour at pH 4. 0 at 60°C. The enzyme was shown to be homogeneous by polyacrylamide gel electrophoresis. Akrilex C-100, a polyacrylamide bead polymer containing carboxylic functional groups (6. 4 meq/g solid), was a commercial product of Reanal Factory of Laboratory Chemicals (Budapest, Hungary). Carbodiimides were synthetized according to JÁSZAY et al. (1987). Soluble starch was purchased from E. Merck GmbH (Darmstadt, Germany). All other chemicals were reagent grade commercial preparations of Reanal.

Immobilization: Akrilex C-100 xerogel (1 g) was suspended and swollen in 50 ml of 0.1 M potassium phosphate buffer (pH 7.5). A water-soluble carbodiimide, in a stoichiometric quantity relative to the carboxylic functional groups located on the support, dissolved in 25 ml of cold (0°C) buffer, was added, with continuous stirring and cooling in an ice bath. After 10 min, 25 ml of enzyme solution containing 0.5g of protein was added, and the pH was adjusted to 7.5. The mixture was incubated at 0–4°C for 48 h, with two 6-h periods of agitation. The gel was filtered by suction and successively washed three times with 100 ml of buffer, three times with 100 ml of buffer containing 1.0 M sodium chloride and three times with 100 ml of buffer to remove unbound proteins, and finally, with a large volume of distilled water to remove the buffer ions. The products were lyophilized.

Protein measurements: Protein was determined according to the method of LOWRY et al. (1951) as modified by SCHACTERLE and POLLACK (1973). The amount of immobilized protein was calculated from the difference between the amount of protein introduced into the reaction mixture and the protein present in the filtrate and washing solution after immobilization (SZAJÁNI et al., 1980).

Assay of glucoamylase activity: In the activity testing of the soluble enzyme, the reaction mixture (5.1 ml) contained 40 mg/ml soluble starch (pH 4.0) and 5–12 μg/ml enzyme. After 30–90 min at 60°C , the reaction was terminated by alkali treatment. The control containing only substrate was treated in an identical manner. In the case of immobilized glucoamylases, 1.5–2.0 mg of immobilized enzyme (dry) suspended in 5.0 ml 40 mg/ml soluble starch at the optimum pH for the catalytic activity was stirred for 45–120 min at 60°C. The enzyme was then filtered off quickly (a few seconds) and the concentration of liberated D-glucose was determined by iodometric titration (ERDEY, 1956.).

Thin-layer chromatography: For the thin-layer chromatography of saccharified starches, HPTLC aluminium sheets (20x20 cm) with a silica gel 60F layer were purchased from E. Merck. The sheets were developed in a Chrompress 10 pressurized ultramicro chamber (Labor MIM, Budapest, Hungary). The concentration of the saccharified starches and the standard was 0.1% (w/v) in water. Each spot represents about 3 μg of carbohydrate. The chromatoplates were developed in an acetonitril-water 1:2 (mol/mol) solvent system. The development required 20–25 min at room temperature. The membrane pressure was 1.2 Mpa. After drying at 80°C for 5 min, the carbohydrates were identified with a staining reagent composed of 50 ml of solution A+50 ml of solution B Solution A contained 2 g of diphenylamine and 2 ml of aniline in 50 ml of acetone, and solution B 10 ml of phosphoric acid (85%) diluted to 50 ml with deionized water.

Results and discussion

As regards the activity on a dry weight basis, the application of N-*t*-butyl-N′-dimethylaminopropyl carbodiimide methyl iodide (BDAPI) as coupling agent proved to be most favourable, but the highest activities on a protein basis were experienced for BDAPI and N-ethyl-N′-dimethylaminopropylcarbodiimide methyl chloride (EDAPC).

Table 1. Effects of carbodiimides as coupling agents on catalytic activity of immobilized glucoamylases

Carbodiimide	activity on a dry weight basis (unit/g)	activity on a protein basis (%)
BDAPI	41. 6	10. 0
CMC	17. 7	5. 0
EDAPC	13. 5	13. 0

[a] The activity of the soluble enzyme was taken as 100 %.

pH dependence of catalytic activity: The pH dependence of the initial rate of hydrolysis of soluble starch by immobilized glucoamylases was studied at the same ionic strength in the pH range from 2.5 to 5.5 (Fig. l). The curves for the immobilized glucoamylases produced with EDAPC and BDAPI as coupling agents were practically identical, with a maximum value at pH 4.5. The immobilized glucoamylase prepared with N-cyclohexyl-N′-morpholinoethyl carbodiimide metho-*p*-toluenesulfonate displayed a somewhat different function, with a maximum at pH 4.0, which is the same as that for the soluble enzyme (SZAJÁNI et. al., 1985).

Dependence of catalytic activity on temperature: The temperature dependence of the activity of the immobilized glucoamylases was studied in the temperature range 40 to 70 oC at the optimum pH for the catalytic activity. Initial velocities were derived from the amount of D-glucose liberated from soluble starch during a 60-min incubation at selected temperatures (Table 2.).

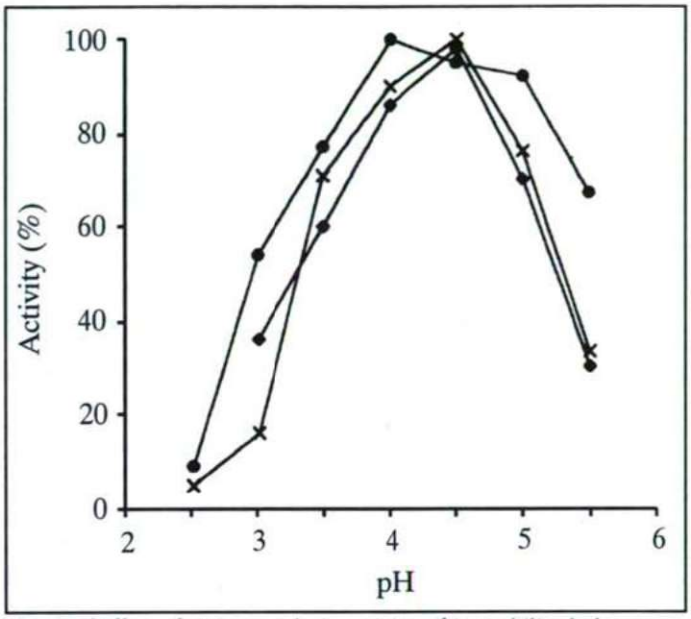

Figure 1 Effect of pH on catalytic activity of immobilized glucoamylases prepared with different disubstituted carbodiimides as coupling agents. Experiments were performed at 60°C. ♦, BDAPI; ●, CMC; x, EDAPC. The maximum activities were taken as 100%.

Table 2. Dependence of catalytic activity of immobilized glucoamylases on temperature.

Temperature (oC)	Carbodiimide		
	BDAPI	CMC	EDAPC
	Activity (%) [a]		
40	54.1	40.9	56.8
50	70.9	77.4	86.6
60	100.0	100.0	100.0
70	27.0	36.4	31.8

[a]The activity measured at 60°C was taken as 100%.

The data indicated that the temperature dependence of the catalytic activity of the immobilized glucoamylases produced by using various disubstituted carbodiimides differed somewhat.

Starch splitting: The catalytic activity of the immobilized glucoamylases was studied with a thinned starch substrate (1.2 g/ml) containing glucose, maltose, maltotriose, maltotetraose and short — chain dextrins. After incubation for 60 h at 60°C, the reaction was stopped by filtration (a few seconds) and the samples were analysed by means of thin-layer chromatography (Fig. 2). Surprisingly, significant differences could be detected reflecting different rates of hydrolysis in the starch splitting patterns. The immobilized glucoamylases produced with EDAPC and BDAPI as coupling agents showed similar splitting patterns, which differed from that of the immobilized glucoamylase produced with CMC, this resembling the splitting pattern of the soluble enzyme. It is presumed that the differences are caused by the different spatial localization of the enzyme molecules in the support matrix as a special effect of the carbodiimide structure resulted in different steric hidrances. Some changes in the mode of action of the immobilized glucoamylases can not be excluded.

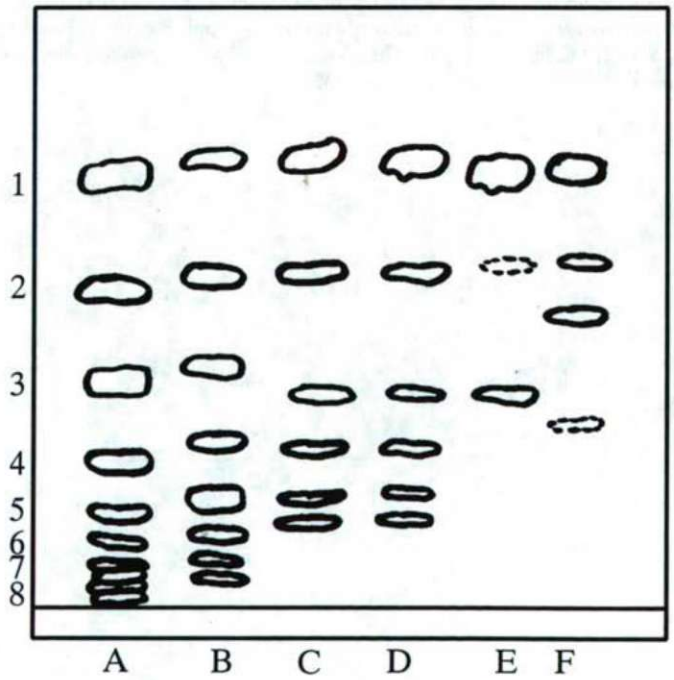

Figure 1 Thin-layer chromatography of starch splitting products. A, Standard; B, thinned starch. Thinned starch processed by glucoamylase immobilized with C, EDAPC; D, BDAPI; E, CMC; F, thinned starch processed by soluble glucoamylase. 1: glucose; 2: maltose; 3: maltotriose; 4: maltotetraose; 5: pentose; 6: hexose; 7: heptose; 8: octose.

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HYDROGENASE ACTIVITY ASSAYS ON *METHYLOCOCCUS CAPSULATUS* (BATH)

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Abstract

The presence and activity of hydrogenase in the thermotolerant methanotroph *Methylococcus capsulatus* (BATH) was demonstrated. Hydrogenase activity was found by two independent enzyme assays in cells cultivated under routine methanotroph-growing conditions. Hydrogenase activity without prior induction by molecular hydrogen or nitrogen fixing growth suggests that a constitutively expressed hydrogenase is present in *Mc. capsulatus* (BATH). Hydrogen utilising activity reached half of the maximal value at 2% hydrogen in the headspace. Molecular hydrogen was suitable to provide reducing power for the sMMO in TCE degradation via the hydrogenase.

Key words: Biotechnology, Hydrogenase, Methane monooxygenase, Biohydrogen, Methanotrophic bacteria, Thermotolerance, *Methyloccoccus capsulatus* (Bath).

Introduction

Methane oxidising bacteria (methanotrophs) have attracted considerable interest over the past twenty years due to their potential in producing bulk chemicals, such as propylene oxide, single cell protein and for use in biotransformation (DALTON et al., 1995). More recently, their ability to degrade the groundwater pollutant trichloroethylene (TCE) and other chlorinated compounds has also been examined (OLDENHUIS and JANNSEN, 1993). Methanotrophs are unique in that they only grow on the one-carbon compound, methane. Some will also grow on methanol. They cannot use heterotrophic/multicarbon compounds as sole carbon and energy

sources. Methanotrophs are also an important group of microorganisms as they are a major sink for biologically produced methane in the biosphere and appear to be ubiquitous in nature. They have been isolated from freshwater and marine environments, soils and sediments (BOWMAN et al., 1993) and a number of different genera and species now exist in culture. Despite the widespread nature of methanotrophs in the environment and their biotechnological potential, the thermophilic isolates (> 50°C) have received surprisingly little attention.

Methane is oxidised by methanotrophs using the enzyme methane monooxygenase (MMO). Further knowledge of this enzyme will aid the design of catalysts and development of biocatalysts crucial for the effective use of methane as a fuel and industrial feedstock. MMO exists in two forms in the cell, depending on the availability of copper in the environment (STANLEY et al., 1983). The soluble enzyme complex (sMMO) is present in some but not all methanotrophs. It has been extensively studied in *Methylococcus capsulatus* (BATH), *Methylosinus trichosporium* OB3b and *Methylocystis* strain M (DALTON, 1992; LIPSCOMB, 1994; NAKAJIMA et al., 1992). The sMMO is very unusual in that it will also cooxidise a wide variety of aliphatic, aromatic and halogenated hydrocarbons (COLBY et al., 1977), making it an extremely versatile enzyme for biocatalysis and biodegradation processes. *Methylocystis* strain M is being extensively used in Japan for bioremediation of TCE contaminated groundwater and for biodegradation of TCE (OKADA et al., 1995). The genes encoding the sMMO from *Ms. trichosporium, Mc. capsulatus* and *Methylocystis* strain M have been cloned, sequenced and extensively characterised at the molecular level (MURRELL, 1992, 1994; MCDONALD et al., 1997).

The other form of MMO, found in all methanotrophs, is the membrane bound or particulate form (pMMO). This has proved extremely difficult to purify in active form (NGUYEN et al., 1996). It consists of at least two membrane associated polypeptides which have recently been solubilised in an active state (SHIEMKE et al., 1995). It has a narrower substrate specificity than sMMO does, but has been shown to oxidise trichloroethylene (TCE) albeit at lower levels than sMMO (DISPIRITO et al., 1992). The genes encoding this enzyme have also been cloned and are currently being studied at the molecular level (SEMRAU et al., 1995; HOLMES et al., 1995).

Both MMO enzymes require reducing equivalents for their catalytic activity. Under physiological conditions this is supplied by the oxidation of the methanol produced. Since biodegradation processes such as the decomposition of chlorinated aliphatic compounds by MMO are cooxidation processes, alternative ways of supplying reducing power are needed. The cleanest and economically most promising alternative is the use of hydrogen.

Hydrogenases are metalloenzymes that catalyse the reversible oxidation of molecular hydrogen and, as such, are important enzymes in anaerobic metabolism of both chemotrophic and phototrophic bacteria (VIGNAIS et al., 1995). Because the

enzyme is involved in producing hydrogen from water, nitrogen fixation, biogas production, corrosive sulphate reduction, and specific hydrogenation reactions, it has generated considerable interest as a biological catalyst involved in reactions of major commercial importance.

All hydrogenases contain FeS clusters of various types, but only a small group of enzymes containing only FeS clusters have been characterised. The majority of known hydrogenases have Ni, and a few enzymes contain Ni and Se, in addition to the FeS redox clusters.

Very little is known about hydrogenases in methanotrophs. DEBONT (1976) reported hydrogen uptake activity in *Methylosinus* strain 41. This activity was induced by nitrogen fixing growth conditions only. The presence of an uptake hydrogenase was concluded from the fact that acetylene reduction by whole cells could be driven by molecular hydrogen. Constitutive hydrogen evolving activities from formate under anaerobic conditions were reported for *Methylomicrobium album* BG8 and *Ms. trichosporium* OB3b (KAWAMURA et al., 1983). Maximum activities were 1.5 and 0.45 nmoles hydrogen formed/min x mg dry cell for *Mm. album* BG8 and *Ms. trichosporium* OB3b, respectively. TAKEDA (1988) showed that *Methylocystis* T-1 produced hydrogen under nitrogen fixing growth conditions in the presence of 1.5-5.0% O_2 in the headspace. Nitrogen fixation was inhibited at higher oxygen concentrations, whereas no hydrogen was detected when the ratio of oxygen was decreased below 1.5%. These results suggested the presence of an uptake hydrogenase sensitive to oxygen concentrations exceeding 1.5%. CHEN and YOCH (1987) reported distinct constitutive and inducible hydrogen uptake activities in *Ms. trichosporium* OB3b. The constitutive activity was observed under all growth conditions tested and had a v_{max}=60 nmoles hydrogen consumed/min x mg dry cell, and a very high K_M of 50% for H_2. Induction of the inducible activity could be achieved in mature cells by overnight incubation under an atmosphere of 50% hydrogen, 5% air and 45% argon in the absence of methane and ammonia. The inducible activity had a v_{max}=32 nmoles hydrogen consumed/min x mg dry cell, and a distinctly lower K_M of 1% for H_2. The hydrogen uptake activity in *Ms. trichosporium* OB3b was shown to be able to supply reducing power for both sMMO and pMMO activities (SHAH et al., 1995).

MATERIAL AND METHODS

Cultivation of organisms

Unless otherwise indicated, strains were grown in NMS medium (WHITTENBURY and DALTON, 1981) containing 0. 4 μM $CuSO_4$, 7. 5 μM $NiCl_2$ and 16 μM $NaMoO_4$. VCR NMS medium consisted of the above medium supplemented with vitamins (KANAGAWA et al., 1982). To solidify media, 1. 5 % (w/v) of Bacto agar (Difco Laboratories) was routinely added. Liquid cultures were grown in 15 or 100 ml of medium in 50 or 500 ml conical flasks shaken at 200 rpm. Flasks were stoppered with rubber

Suba Seals. Head space was filled with a methane:air:CO_2=50:48:2 gas mixture. The gas-to-liquid ratio in the bottles and flasks was 4:1. Incubation temperature was 43°C. Liquid cultures were also grown in a „BioFlo IIC" New Brunswick fermenter equipped with 1.5 l glass vessel. Operational conditions of the fermenter were 40°C, 150–250 rpm agitation, pH 6.8, continuous addition of methane and air at 75 and 50 ml flow rates, respectively. Cell density was kept between OD_{540}=1.0–2.0 via continuous fermentation. Plates were incubated in anaerobic jars under the same methane-air-CO_2 mixture for 10–15 days. The gas phase was replaced every 4–5 days with the methane-air-CO_2 mixture.

Hydrogen evolving activity assay with whole cells

1 day old batch cultures of *Mc. capsulatus* (BATH), OD_{540}=0.25–0.50 were concentrated by centrifugation (10.000 rpm, 15 mins) and resuspended in 20 mM potassium phosphate buffer (pH 7.0) to give a final density of OD_{540}=1.0–2.0.2 ml of the resulting biomass were added, together with 1 ml 2 mM methyl viologen, to a special reaction vessel of 30 ml in volume. The reaction vessel enabled the replacement of the headspace with nitrogen, as well as, the prior addition of sodium dithionite into a separate compartment. After replacing the headspace with nitrogen, contact between sodium dithionite and the reaction mixture was established, resulting in the quick reduction of methyl viologen associated with the appearance of dark blue colour. This was considered as the zero time point of the reaction and incubation was carried out at 43°C. 500 µl of headspace were analysed after 20 min on a Hitachi 263–50 gas chromatograph. Operational parameters of the GC were as follows: 2 m long column of 1. 5 mm in diameter filled with 5 Å Molecular sieve. Injector, column and detector areas of the gas chromatograph were heated to 120°C. Nitrogen was used as the carrier at 50 ml/min flow rate.

All results represent the average of three separate assays.

Hydrogen uptake activity assay with whole cells

Fermenter grown cultures were concentrated and resuspended in 20 mM potassium phosphate buffer (pH 7.0) to give a final density of OD_{540}=1.0. Redox dye was added to the resulting cell suspension to give a final concentration of 0.2 mM for methylene blue (MB), 0.25 mM for methyl viologen (MV) and 0.4 mM for benzyl viologen (BV). 2 ml of cell suspension were added to anaerobic cuvettes of 5 ml total volume, stoppered with rubber Suba Seals. After replacing the headspace with nitrogen and 2 min of preincubation at 43°C, reactions were started by replacing a given percentage of the headspace with hydrogen. A Unicam UV/VIS UV2 spectrophotometer, equipped with a heated (to 42°C) multiple cell holder, was used to follow the reaction at 600 nm, in the case of methyl- and benzyl viologen and at 650 nm in case of methylene blue. The Vision/Rate and Spserv softwares were used to analyse the results. All results shown represent the average of at least three separate assays.

Hydrogen driven TCE degradation by whole cells

Mc. capsulatus (BATH) was grown in the fermenter in copper free NMS medium to a density of OD_{540}=5.5 to enable the expression of the sMMO. Bacteria were concentrated by centrifugation and resuspended in 20 mM potassium phosphate buffer (pH 7.0) to give a final density of OD_{540}=10.0. The reaction mixture (in a 25 ml conical flask) consisted of 4.0 ml 20 mM potassium phosphate buffer; 500 µl 1 mM TCE stock solution (aqueous) and 100 µl of 1 M sodium formate, if added (in which case, the volume of the phosphate buffer added was reduced correspondingly). 10 ml of the headspace was replaced by hydrogen or methane for hydrogen and methane driven TCE degradation assays, respectively. The reaction was started after 1 min of preincubation at 42°C by the addition of 500 µl cell suspension. The flasks were shaken at 200 rpm. 500 µl samples were withdrawn after 1 min and extracted with 500 µl n-pentane. 1 µl of the organic phase was then analysed on an SRI 8610C gas

chromatograph equipped with electron capture detector. 50 μM dibromoethane was used as an internal standard. Operational parameters of the gas chromatograph were: T_{column} = 50°C to 130°C in 3 mins; $T_{detector}$ = 200°C; $T_{injector}$ = 150°C; nitrogen carrier at 9 ml/min flow rate; 30 m x 0.53 mm capillary column filled with MXT-VDL (Restek Corp.).

Results and Discussion

Hydrogen evolution by Methylococcus capsulatus (BATH)

Hydrogen evolving activity was demonstrated from reduced methyl viologen at 108 ± 30 nmol hydrogen produced/min x mg dry cell activity. Hydrogen production at this rate by *Methylococcus capsulatus* (BATH) without prior induction indicated constitutive expression of the corresponding enzyme. In order to obtain further evidence for this hypothesis we carried out hydrogen uptake assays.

Hydrogen uptake assays

Even though there are several hydrogen utilisation assays described in the literature, hydrogen uptake prove very difficult to measure in methanotrophs. Part of the reason for this is that methanotrophs tend to store energy, mainly in the form of polyhydroxyalkanoates (PHA). PHA is metabolised during the assay and the derived reducing power is used to reduce the redox dye. Thus, a hydrogen free negative control is very likely to show some activity and this has to be subtracted from the result of the actual assay.

Assays with oxidised methyl viologen as the electron acceptor required a slight pre-reduction of methyl viologen. This was necessitated by the fact that the oxidised form of the redox dye was unable to cross the cytoplasmic membrane, as was found for *Thiocapsa roseopersicina* (BAGYINKA et al., 1983). The low standard redox potential of methyl viologen (–446 mV), however, enabled the production of hydrogen from the reduced portion of the dye. Production of hydrogen was demonstrated by gas chromatography. Consequently, subtracting the result of the negative control from that of the hydrogen containing assays resulted in a combination of hydrogen uptake and hydrogen production rates. This made reliable analysis of the results impossible.

Methylene blue was used as a redox dye because of its relatively high (+11 mV) standard redox potential, which does not allow the production of hydrogen from reduced methylene blue. However, due to this high standard redox potential, methylene blue is prone to be reduced by most redox systems of the investigated bacteria, resulting in a very high background activity. In many cases, this background activity was higher than the actual hydrogen uptake activity we tried to measure.

Benzyl viologen has a standard redox potential of -350 mV, slightly higher than that of methyl viologen. Fortunately, there was no need for pre-reduction of benzyl viologen for the hydrogen uptake assay. Moreover, the -350 mV standard redox potential was still too high for most of the redox systems of *Methylococcus capsulatus* (BATH), thus the problems encountered with methylene blue did not appear.

Hydrogen uptake rates from batch cultures of *Mc. capsulatus* (BATH) were not reproducible. Our hypothesis for this phenomenon is that exponentially growing batch cultures of methanotrophs use all the provided methane within 6 to 8 hours under the conditions applied. Thus, depending on the viability of the inoculum, an overnight batch culture of *Mc. capsulatus* (BATH) uses all the available methane 2 to 8 hours before harvesting and subsequent assaying. This may result in bacteria with highly variable physiological conditions (i.e. still very close to the exponential

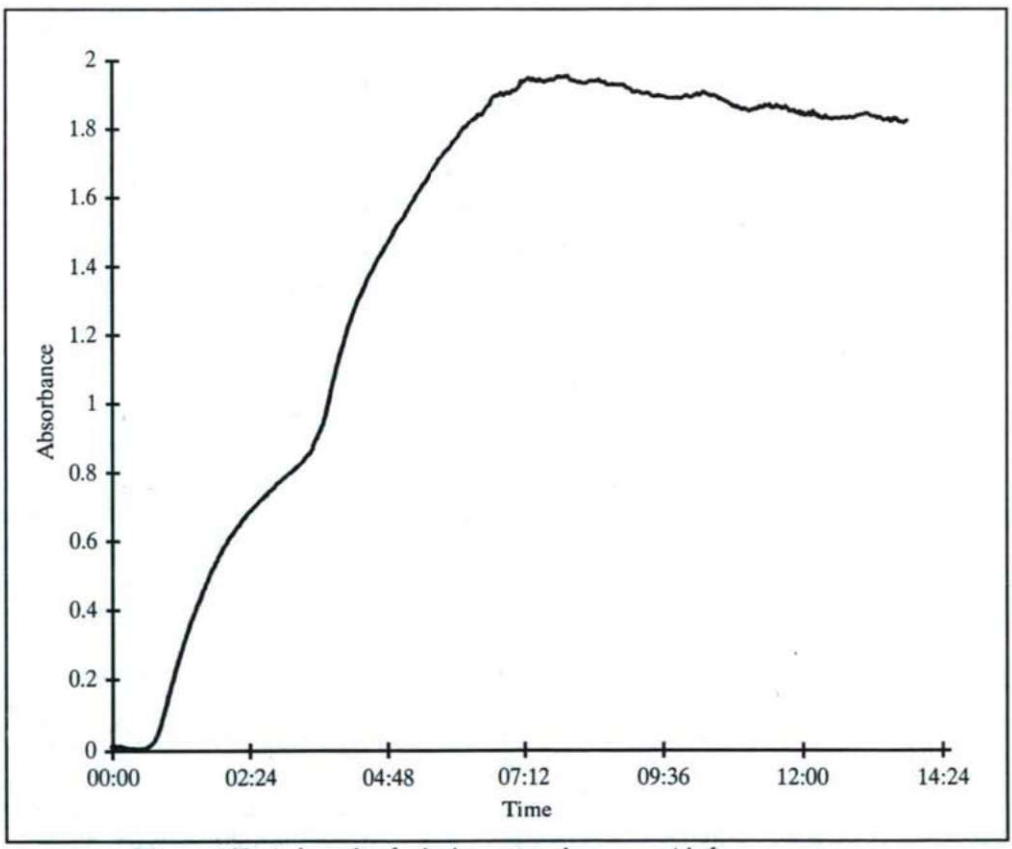

Figure 1 Typical result of a hydrogen uptake assay with fermenter grown *Methylococcus capsulatus* (Bath). The headspace contained 2% hydrogen.

phase or already in the late stationary phase of growth) between different assays. Continuous fermentation of methanotrophs reproducibly provided biomass in the exponential phase.

Hydrogen uptake activity showed a complex curvature (Fig. 1). The first part showed no dependence on the concentration of hydrogen in the headspace and activity values calculated from this curve were not reproducible. Thus all the activity data are derived from the second section of the hydrogen uptake curves.

Hydrogen uptake by Methylococcus capsulatus (BATH)

Hydrogen uptake measurements were carried out (applying the above described optimised assay with benzyl viologen on fermenter cultures of *Mc. capsulatus* (BATH)) under different concentrations of hydrogen in the headspace. Results (shown in Figure 2) indicate the constitutive expression of a hydrogenase with a K_M value for hydrogen of approximately 2%. Constitutive expression and high affinity for hydrogen are features which may be very useful for biotechnological applications of the hydrogen utilisation capability of *Mc. capsulatus* (BATH) (Fig. 2).

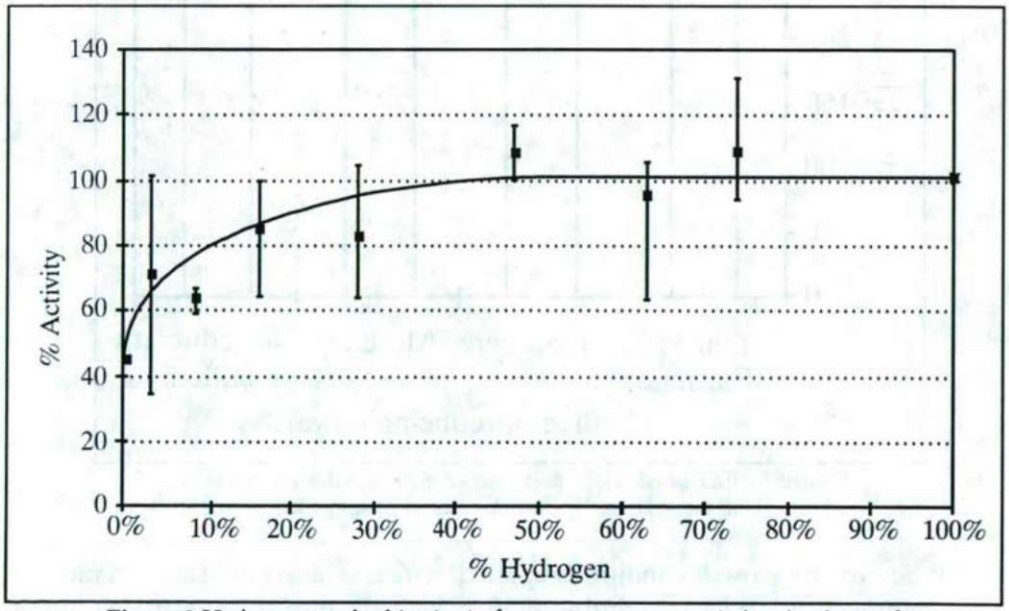

Figure 2 Hydrogen uptake kinetics in fermenter grown, non-induced culture of *Methylococcus capsulatus* (Bath).

Hydrogen driven TCE degradation by Methylococcus capsulatus (BATH)

To demonstrate the biotechnological potential of the hydrogen uptake activity of *Mc. capsulatus* (BATH), TCE degradation assays were carried out with various reducing sources (Fig. 3). There was a significant background activity (no reductant

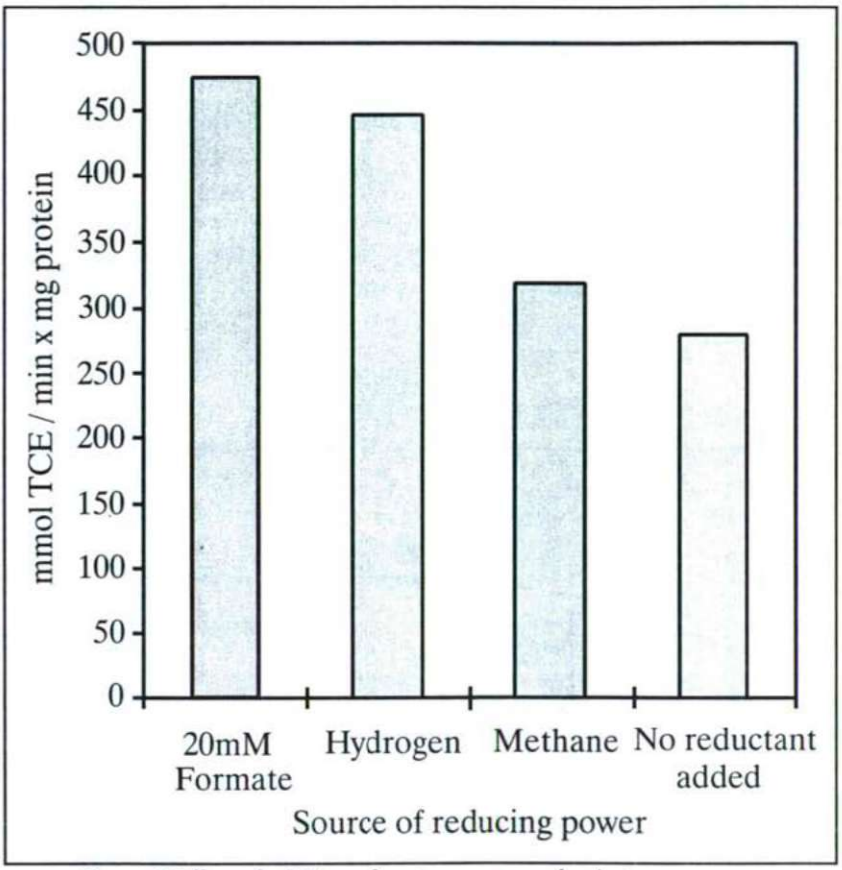

Figure 3 Effect of addition of various sources of reducing power on TCE degradation by *Methylococcus capsulatus* (Bath).

added) due to the growth conditions applied, which enabled the accumulation of high amounts of PHA. The addition of 50 % methane into the headspace did not increase the TCE degradation rate significantly. This is probably due to the competitive inhibition of TCE oxidation by methane, which was almost balanced by supplying reducing power via further oxidation of the methanol produced.

Addition of sodium formate did, however, cause a significant increase in TCE degradation, as found by many other authors. The effect of 50 % hydrogen in the headspace was comparable to that of sodium formate, with only a very slight difference. This result demonstrated the applicability of hydrogen uptake activity of *Mc. capsulatus* (BATH) in driving whole cell sMMO activity for the biodegradation of recalcitrant chlorinated hydrocarbons.

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WHY ARE ETHYLOESTRADIOL AND D-NORGESTREL CAPABLE OF EMERGENCY CONTRACEPTION?

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Abstract

The author investigated the effects of 0.05mg ethyloestradiol and 0.25mg d-norgestrel on endrometrium. He established that this combination is capable of emergency contraception, because it prevents the implantation of the fertilized ovum.

Key words: emergency contraception, contraception for young people, endometrium.

Introduction

With optimal administration of d-norgestrel no cells of chorial origin were detectable in the pre-menstruation histological samples. Moreover, no sign of secretion or predecidual reaction was seen (FARKAS et al., 1982).

Serum progesteron levels measured on the 21. day of the cycle proved that in most cases the drug can have an ovulation-inhibitory effect even at optimal administration, therefore, the prevention of nidation was not possible (FARKAS et al., 1982).

The drug can be taken as effective for post-coital contraception even at low dosage and in the case of multiplied intercourses (FARKAS and SAS, 1984).

A sperm needs 90 seconds to get to the cervix, while the inhibition of the penetration of sperms develops after 3 hours of administration of the drug (KESERŰ and PARRADA, 1975).

Frequent occurrance of extrauterine pregnancy indicated the tubamobility-decreasing effect of the drug (FARKAS, 1994).

In order to reduce the side effects, we proposed to bring out a contraceptive drug with low estrogen content (FARKAS et al., 1981, FARKAS and SAS, 1984, FARKAS, 1978).

In the course of contraception with Ovidon (0.05 mg ethyloestradiol and 0.25 mg d-norgestrel) we observed a shortening of the proliferation period without presecretion period to occur (OUTLOOK..., 1966).

Prolonged administration of Ovidon pills frequently led to functional infertility (GOLDZICHERT et al., 1964).

The clinico-pharmacological studies carried out so far indicate that the active compounds of Ovidon can be used, in addition to the inhibition of ovulation, for the inhibition of nidation, as well (FARKAS, 1979, FARKAS et al., 1976).

Yuzpe (FARKAS and KAJTÁR, 1996) reported about the success of com-bined administration of de-norgestrel and ethyloestradiol.

Material and methods

Changes in the endometrium were analysed following administration of Ovidon pills (0.05 mg ethyloestradiol and 0.25 mg sd-norgestrel). Therefore, 15 patients underwent a stripe-curettage between the 16th and 25th day of the cycle.

The small pieces of corpus mucosa obtained from the curettage were transferred immediately into 10% (v/v) neutral formaline. Duration of the fixation was usually 20 to 24 hours. The fixed and cleaned samples were then embedded in paraffin and series of slices were produced from them.

The series of slices are useful to detect changes in any histological sample, even if they are best visible at another cutting plane.

After deparaffinization and dehydration, the samples were investigated by light microscopy after haematoxylin-eosin staining following a routine histological operation. The histological samples were compared with those obtained in the physiological and secretion period, as well as with histological alterations on the effects of high-dose drugs from our own earlier samples and from literature observations.

Results

In those cases where the cycle was built up by Ovidon administration (a relatively short duration of drug treatment) significant alterations were detected in the endometrium, as compared with that of normal cycle.

Several glands were found to exhibit atrophy of differing extent. Some of them became narrow, others became large, sometimes they became microcystic and their lumen became empty. These are usually lined by a single layer of cuboidal epithelial cells.

These glands which are lined by different types of epithelia can be found next to each other quite often. In some glands even flattened, endothel-like epithelial lining can also be observed.

In several cases small vacuoles are seen near the nuclei which contain glycogen by PAS staining. These vacuoles are very variable in location, they do not show the regularity which is detectable in the corresponding period of the normal cycle and only in several glands or gland epithelium.

The presence of glycogen is anticipated mainly in those cases where the progesteron derivative is in excess. In no cases was glygogen detected in the lumen. In the second part of the cycle, no mitosis can be seen in the cells of gland epithelium.

The focal oedema of the stroma is well detectable. The oedematous, loose areas in the neighbourhood of densely packed regions are especially well visible (Fig. 4/a).

It is well seen that the ratio of glands to stroma is shifted in favour of the stroma. No expressed predecidual transformation of the stroma cells was observed, indicating that the progestogene component of the drug was not dominant.

Differentiation of stroma cells was not detectable following administration with Ovidon in these short administration periods. Predecidual transformation took place only in small areas, although these areas contain relatively large number of

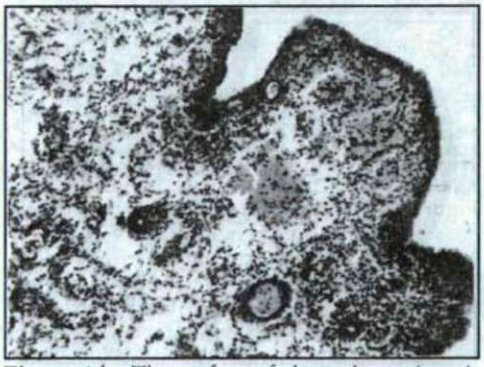

Figure 1/a. The surface of the endometrium is uneven. The stroma contains signs of focal oedemas and bleedings. The glands are tight, their epithelia are not secernating.

Age: 24 years. Last normal menstruation: 15 June. Duration of drug admi-nistration: 2 years. Date of biopsy: 9 July (24th day)

Quantity of drug administered during the cycle: 4.25 mg d-norgestrel and 0.085 mg ethyloestradiol. Obstetrical events: obstesy: 1, abortion: 0. Last obstetrical event: obstesy on 15 May, 1981.

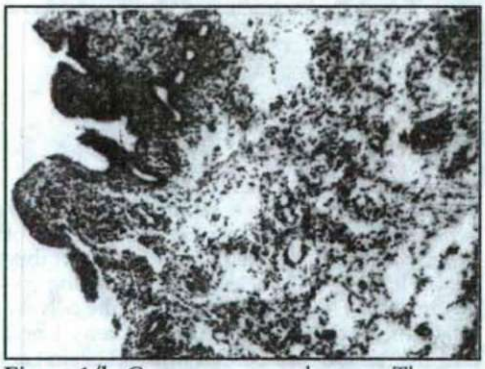

Figure 1/b. Corpus mucosa polyposus. The stroma is loosened, narrow inactive glands are visible in it, their number is reduced. Stroma cells are slightly swollen.

Age: 24 years. Last normal menstruation: 15 June. Duration of drug administration: 2 years. Date of biopsy: 9 July (24th day)

Quantity of drug administered during the cycle: 4.25 mg d-norgestrel and 0.085 mg ethyloestradiol. Obstetrical events: obstesy: 1, abortion: 0. Last obstetrical event: obstesy on 15 May 1981.

endometrial granulocytes. In the other areas of the stroma, however, no sign of cellular differentiation can be seen.

The reticulum fibre network of the stroma is variable. It is well developed in the densely packed areas, whereas it is underdeveloped in the loose regions.

The spiral arteries are usually underdeveloped: only small, linear capillaries can be seen, with dilatated thin-walled arteries in other areas (Figs. 4/b and 4/c).

Long-term treatment (up to several years) resulted in a surprising histological pattern. Abortive secretion was seen only occasionally in the glandular epithelium (Figs. 1/a and 1/b).

The number of glands is decreased from cycle to cycle, finally they disappear or only their residues can be seen. These residues are glands with endothel-like epithelium lining which can be easily mistaken for the capillaries (Figs. 2/a, 2/b, 2/c and 2/d).

The stroma does not exhibit atrophy in all cases. Decrease in the number of stroma cells and their replacement by collagen was detectable only focally (Figs. 3/a and 3/b).

Figure 2/a. The surface of the mucous membrane segment is uneven, the number of glands is reduced, their lumen is narrow, they are lined by one-nucleus non-secernating epithelium. The stroma is in excess, the cells are slightly swollen, its structure is loose. 1 or 2 thick arteries can be seen instead of spiral arteries.

Age: 33 years. Last normal menstruation: 16 June. Duration of drug administration: 2 years. Date of biopsy: 12 July (25th day).

Quantity of drug administered during the cycle: 4.5 mg d-norgestrel and 0.090 mg ethyloestradiol. Obstetrical events: obstesy: 3, abortion: 1. Last obstetrical event: obstesy on 30 November 1981.

Figure 2/b. Only tight inactive glands cen be seen. The stroma is in excess.

Age: 33 years. Last normal menstruation: 16 June. Duration of drug administration: 2 years. Date of biopsy: 12 July (25th day).

Quantity of drug administered during the cycle: 4.5 mg d-norgestrel and 0.090 mg ethyloestradiol. Obstetrical events: obstesy: 3, abortion: 1. Last obstetrical event: obstesy on 30 November, 1981.

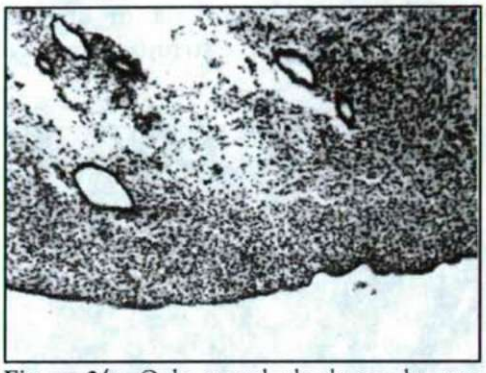

Figure 2/c. Only several glands can be seen which are not secernating and are lined by iinactive epithelium. The stroma cells are slightly swollen, with several thin-walled capillaries between them. No spirtal arteries are developed.

Age: 33 years. Last normal menstruation: 16 June. Duration of drug administration: 2 years. Date of biopsy: 12 July (25th day).

Quantity of drug administered during the cycle: 4.5 mg d-norgestrel and 0.090 mg ethyloestradiol. Obstetrical events: obstesy: 3, abortion: 1. Last obstetrical event: obstesy on 30 November, 1981.

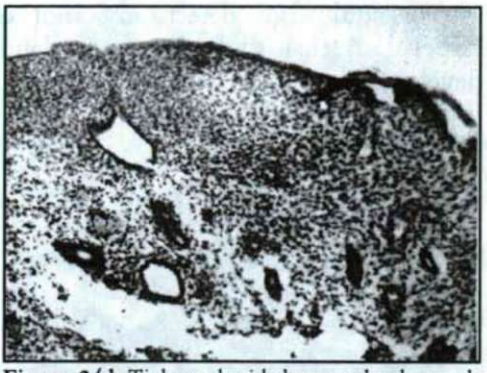

Figure 2/d. Tight and wide-lumen glands are also detectable. The epithelium is not secernating. The stroma is made up of moderately swollen cells. The number of endometrial granulocytes is small. Spiral arteries are not developed.

Age: 33 years. Last normal menstruation: 16 June. Duration of drug administration: 2 years. Date of biopsy: 12 July (25th day).

Quantity of drug administered during the cycle: 4.5 mg d-norgestrel and 0.090 mg ethyloestradiol. Obstetrical events: obstesy: 3, abortion: 1. Last obstetrical event: obstesy on 30 November, 1981.

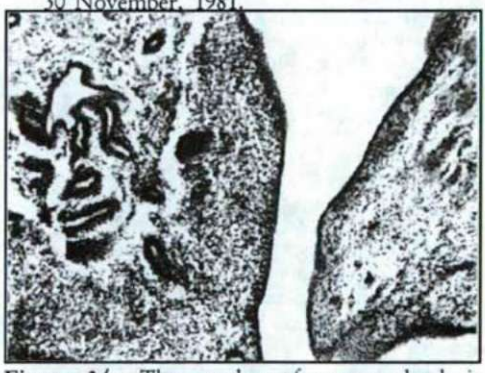

Figure 3/a. The number of corpus glands is small, or they can be observed focally in smaller or bigger groups. The glandular epithelium is inactiove. The stroma predominates. The cells are slightly swollen, or the structure is loosened.

Age: 33 years. Last normal menstruation: 15 June. Duration of drug administration: 7 years. Date of biopsy: 6 July (22th day).

Quantity of drug administered during the cycle: 3.75 mg d-norgestrel and 0.085 mg ethyloestradiol. Obstetrical events: obstesy: 2, abortion: 2. Last obstetrical event: obstesy on 8 December, 1977.

Figure 3/b. In the predominating stroma only very few glands can be seen which are lined by low cylindrical or cubic epithelium. The epithelium is inactive. Several wide-lumen arteries can be seen.

Age: 33 years. Last normal menstruation: 15 June. Duration of drug administration: 7 years. Date of biopsy: 6 July (22th day).

Quantity of drug administered during the cycle: 3.75 mg d-norgestrel and 0.085 mg ethyloestradiol. Obstetrical events: obstesy: 2, abortion: 2. Last obstetrical event: obstesy on 8 December, 1977.

We could not detect any sign of glandularcystic hyperplasia or stroma hyperplasia, that is why no atypic glandular epithelium or atypic stroma cells were developed.

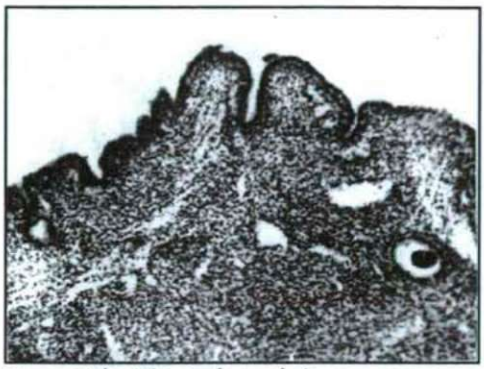

Figure 4/a. The surface of the mucous membrane is slightly uneven. The number of glands is reduced, in some parts secretion of variable extent, mainly abortive, can be seen. In other areas the glandular epithelium is not secernating.

Age: 28 years. Last normal menstruation: 24 June. Duration of drug administration: 1 year. Date of biopsy: 10 July (16th day).

Quantity of drug administered during the cycle: 2.25 mg d-norgestrel and 0.35 mg ethyloestradiol. Obstetrical events: obstesy: 2, abortion: 1. Last obstetrical event: obstesy on 16 June, 1980.

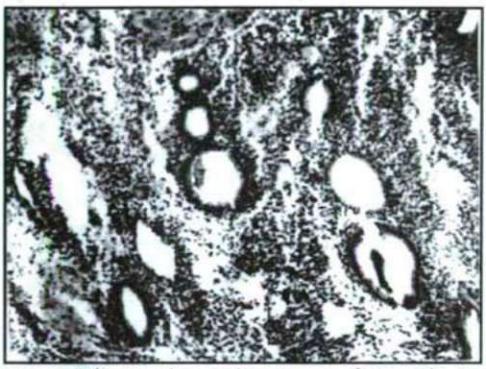

Figure 4/b. Similar to the picture of Fig. 4/B. In the stroma signs signs of focal bleedings and oedema can be seen. The loosening of the stroma is more expressed.

Age: 28 years. Last normal menstruation: 24 June. Duration of drug administration: 1 year. Date of biopsy: 10 July (16th day).

Quantity of drug administered during the cycle: 2.25 mg d-norgestrel and 0.35 mg ethyloestradiol. Obstetrical events: obstesy: 2, abortion: 0. Last obstetrical event: obstesy on 16 June, 1980.

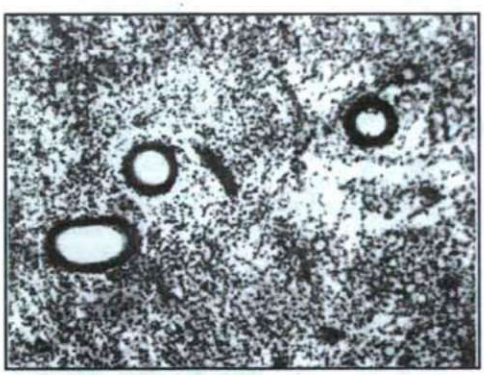

Figure 4/c. The structure of the stroma is loose, in it reduced number of inactive corpus-glands and thin-walled wide arteries can be occasionally seen. No sign of any secretion.

Age: 28 years. Last normal menstruation: 24 June. Duration of drug administration: 1 year. Date of biopsy: 10 July (16th day).

Quantity of drug administered during the cycle: 2.25 mg d-norgestrel and 0.35 mg ethyloestradiol. Obstetrical events: obstesy: 2, abortion: 1. Last obstetrical event: obstesy on 16 June, 1980.

Discussion

After the treatment we observed a gestagene-induced suppression phenomenon, characterized by the shortening of the proliferation period and by a premature reduced secretion activity (FARKAS, 1979, FARKAS et al., 1976, OUTLOOK..., 1966).

The glycogen present in the glandular epithelium was abundant in those cases where the gestagene component was dominant in the applied drug (SIEDEL and HEINEN, 1965).

The majority of the glands became dormant after the 15th day of treatmen. We could not detect any unequivocal sign of predecidual or decidual stroma cell transformation, indicating the harmonized gestagenous action of the drug (OUTLOOK..., 1966).

Dominant gestagenous effect results in predecidual or decidual transformation in an early stage ascompared with the normal cycle, usually between the 15th and 20th days of the created cycle (RYAN et al., 1964, STARUP, 1967, TAYLOR, 1961).

The spiral arteries of the stroma did not develop at all, or if they did, they became small, linear capillaries, sometimes dilatated thin-walled arteries (OBER et al., 1964, OBER, 1966).

Long-term administration resulted in a reduction of the number of glands.

Abortive secretion was detectable only occasionally, it was not present in many cases (KESERŰ and PARRADA, 1975, RYAN et al., 1964).

Such a histological pattern is difficult to distinguish from physiological atrophy, the atrophy present in the endometrium of castrated women (CHARLES, 1964, SHEFFIELD et al., 19691).

During the 9 days until the 16th day of the cycle, altogether 2.25 mg d-norgestrel and 0.35 mg ethyloestradiol were taken up by the patients, which prevented the advantegous physiological changes necessary for the implantation of the morula.

In the early 1980s more than 200 under-18 curettage were performed at the Department of Gynaecology. In 5 month, 11 out of 49 under-18 curettages were necessary because the women had been taking Postinor as a contraceptive drug; 30 out of them employed interrupted intercourse, while 8 had not used any means of contraception.

The 11 curettages mentioned above were carried out on under-16 patients. It can be concluded that administration of Postinor requires rigorous attention as unattentive administration leads to unwanted pregnancy.

Hungarian bibliographical data vary considerably in terms of reliability of the drug, too. The value of the PEARL-index changes between 0 and 12.8 with similar number of patients and cycles. This high variability is not seen in the international literature (variability is between 0 and 5.8), although the active content of the drug is lower there than that of Postinor.

The total dose recommended by YUZPE (YUZPE and LANCEE, 1977) was 200 ng ethyloestradiol and 2 mg dl-norgestrel. 70.4% of the women were treated within 24 hours of the intercourse, 22.7% of them between 24 and 48 hours, and 6% of them were treated between 48 and 72 hours after the intercourse. Of the 464 women having normal cycle the sexual intercourse was in the middle of the cycle. Only 1 pregnancy happened, caused by a mistake of the method, as compared with the minimally expectable 12–30 pregnancies. According to the cumulative rate, 48% of the patients had menstrual bleeding within the first 6 days of the treatment, 71% of them within the first 9 days, 95% of them within the first 15 days and the rest of them within the first 25 days.

In the combined treatment (50 mg ethyloestradiol and 0.25 mg levonorgestrel, also called as Yuzpe-method) the women take 2 pills within the first 72 hours after the intercourse, followed by 2 more pills during the next 12 hours.

The efficiency of the method is highlighted by the fact that following 1 contraception-devoid intercourse only 2% of the women following combined treatment got pregnant, as compared with the 8% of pregnancy of women without using urgency contraceptive pills (OUTLOOK, 1966). This means that the combined treatment reduces the chance of pregnancy by 75%.

During 3 days of administration with Fertilin, altogether 1 mg d-norgestrel and 0.20 mg ethyloestradiol were taken up by the patients. Supposedly the active content in this short period can prevent the physiological changes that are necessary to take place in the stromal and glandular region of the mucous membrane necessary for the implantation of the fertilized ovum. The drug-induced peripheral change taking place during 72 hours does not provide an optimal milieu for the nourishment of the morula, which then dies in a short time. The drug, therefore, can prevent pregnancy by inhibiting implantation.

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INHIBITION OF OVULATION WITH DOPAMIN-ANTAGONISTS

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Abstract

Analysing the results of the Motilium treatment of women in reproductive age different internal medical diseases, the authors discuss the possibility of a new kind of nonhormonal ovulation-inhibitory anticonception. Domperidon preparation have been used as antiemetic and for induction of lactation. This agent increases the seruM prolactin level in early pregnancy, in puerperas and menopause. The results of endocrinologic study of the 3 women treated for gastrointestinal complaints with 3 x 10 mg domperidon are discussed. The analysis of the protein and steroid hormon values determined during treatment, as well as the basal temperature curves indicated anovulatory menstruation. In the authors opinion treatment with dopamine antagonists with subsequent elevation of prolactin level makes the ovulation-inhibitory oral contraceptives unnecessary. They expect that dopamine antagonists will substitute hormonal contraceptives and eliminate the undesirable side-effects of the latter. Cessation of treatment quickly reverses the effect, indicating the reversible character of the increase of prolactin level. The authors also assume that dopamine antagonists will substitute high-dose progesterone presently used for postcoital contraception. Precoital treatment with dopamine antagonists abolishes or postpones ovulation.

Key words: prolactin, dopamine antagonists, hormonal inhibition of ovulation.

Introduction

Combined oral contraceptive tablets introduced more than three decades ago were supposed to solve the problem of birth regulation. However the agents with high level of active substances damaged not only the reproductive system, but also the whole organism.

The use of hormonal contraceptive tablets frequently elicited infertility or damages of the cycle by affecting the endometrial structures or inducing hyperprolactinemia (BROUWERS et al., 1980).

Taking oral contraceptive tablets is a serious risk factor in family planning. Furthermore these tablets also lead to the developement of vascular diseases (COBRIN et al., 1985).

Several new aspects of contraception providing better family planning in future came up.

The Motilium preparation produced by the Richter Pharmacological Works contains 10 mg domperidon and is used as antiemetic and lactation-inducing drug. This drug was found to elevate the serum prolactin level in pregnants, puerperas and women in menopause (FARKAS, 1989; FARKAS et al., 1989).

Can domperidon as a dopamin inhibitor which elevates the serum prolactin level inhibit the ovulation in the physiological cycle?

If the answer is positive, then taking the dopamine inhibitors makes oral contraceptives unnecessary and even deleterious. The wide use of these drugs is expected in neurology and internal medicine.

Pharmacological and biological effects of domperidon

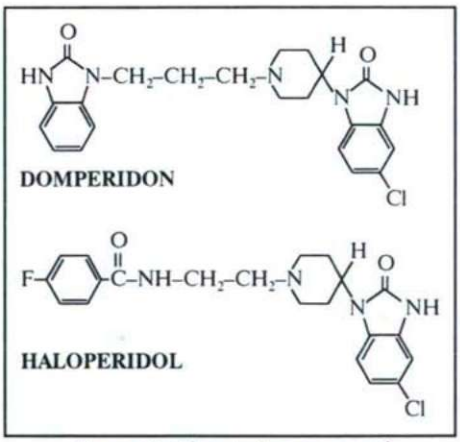

Figure 1. Chemical structure of domperidon and haloperidol.

Chemical structure of domperidon is similar to that of neuroleptics (Fig. 1.). It also elevates the serum prolactin level but does not penetrate via the blood brain barrier. In rare cases this agent induces mastodynia or galactorrhea.

Cessation of treatment with this substance normalizes the serum prolactin level.

Domperidon is a new antidopamine preparation. It stimulates prolactin secretion in hypophysis independently of the applied dose. The domperidon-induced prolactin secretion is higher in luteal than in follicular phase of a normal cycle. It can interfer with the menstrual cycle, but does not affect the fertility in males.

Metoclopramid inhibits both the periferal and the central dopaminreceptors. Following treatment with both agents, there was a 10-fold increase in serum prolactin level. Long-time metoclopramid treatment further elevates the prolactin values (a 25-fold increase), while long-term taking of omperidon decrease the serum

prolactin level below the normal values (a 6-fold decrease) (BROUWERS et al., 1980.).

Dopamine itself is a prolactin-inhibiting factor (PIF). Special very short tubero-infundibular dopaminergic neurons (TIDA) have been found in the vicinity of eminentia mediana (FUXE, 1964).

Somatropine and prolactin have a definite rhythm. The question arises whether it is a real endogenous rhythm?

There is a close relationship between the secretion of these hormones and periods of sleep. Somatotropin secretion is elevated in the early phase of sleep (during the first two hours) and is followed by a marked increase of its serum concentration. By the third hour the concentration of this hormon decreases to 0.

The prolactin rhythm differs from that of somatotropin, for the prolactin level reaches its maximal values by the end of the sleeping period. The oscillation amplitude for somatotropin is about 5 units and for prolactin 1 unit (WISSER and BREUER, 1981).

Materials and methods

We have treated three women of reproductive age for gastrointestinal symptoms (emesis, nausea) and disturbances of gastric motility with daily 3 tables of domperidon (3 x 10 mg). Their first mentsruation occured at the age of 13–14. All the patients were 20–37 years old. They menstruated every 28 days for 4–5 days. One patient have already had 2 children, the rest have not yet been pregnant, but had stable cycle. Thus their fertility can also be considered as a proved. Neither patient took contraceptive drugs, all of them used biological methods.

Fasted venous blood samples have been taken on the day 6 before-, and days 9, 14 and 22 during treatment. Following centrifugation, the serum was stored at -20C°.

Prolactin, FSH, LH and 17-beta-oestradiol levels were determined with RIA (IZINTA MTA KITT).

During the period of treatment all the patients had normal sexual life and did not use contraceptive drugs. During the cycle they measured their basal temperature and plotted temperature curves.

We compared the results of domperidon treatment to those following treatment with three-phasic TRI-REGOL tablets, because the new substance contains less oestrogen.

Results

Compared to the controls, the serum prolactin values on the 22nd day of the cycle were elevated in all three patients. In 2 cases these values exceeded 500 mIU/l (Fig. 2.).

In one case the FSH level increased from 5,3 IU/l to 14,7 IU/l by the 22nd day of the cycle. In two other cases its values remained around 10 IU/l both in the follicular and luteal phases (Fig. 3.).

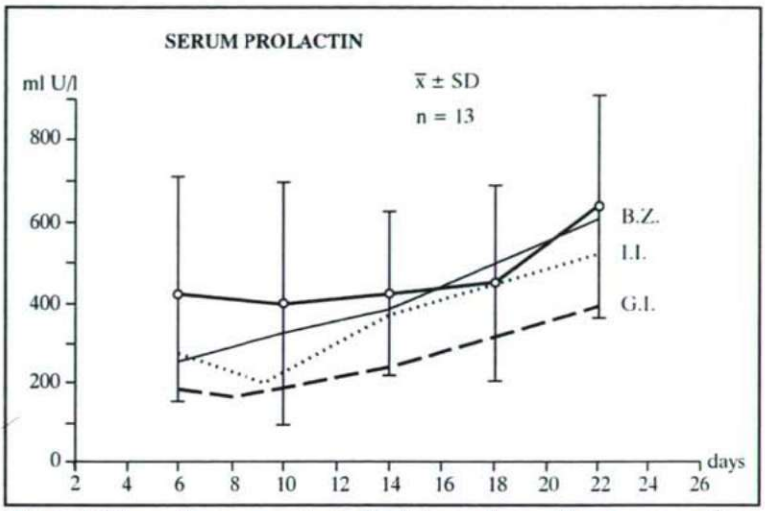

Figure 2. Change in serum prolactin level due to domperidon treatment from day 6 until day 22 of the cycle (n=3). (Before TRI-REGOL treatment x +/-SD, n=13)

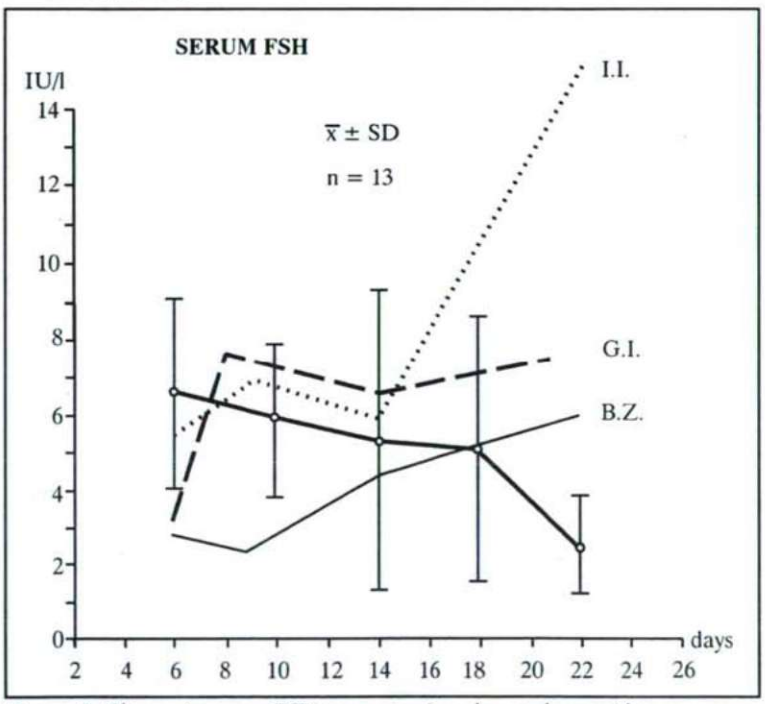

Figure 3. Change in serum FSH concentration due to domperidon treatment from day 6 until day 22 of the cycle. (n=3) (Before TRI-REGOL treatment x +/-SD, n=13)

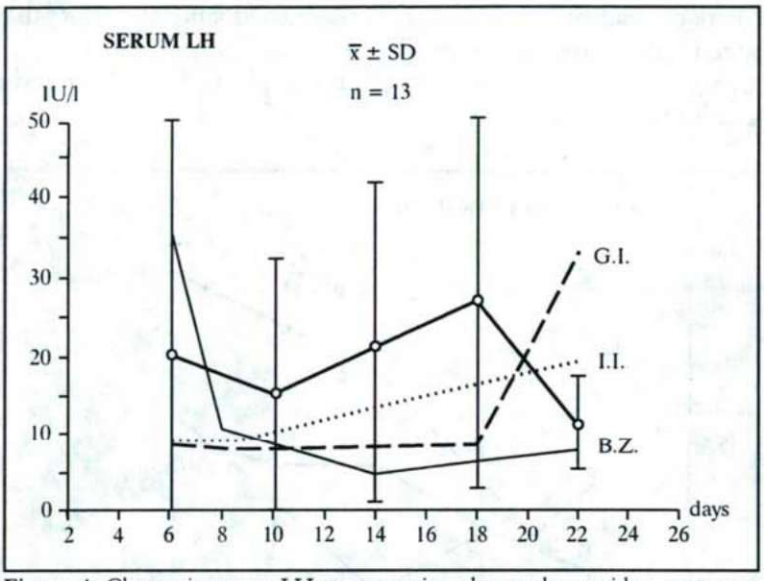

Figure 4. Change in serum LH concentration due to domperidon treatment from day 6 until day 22 of the cycle. (n=3). (Before TRI-REGOL treatment x +/-SD, n=13)

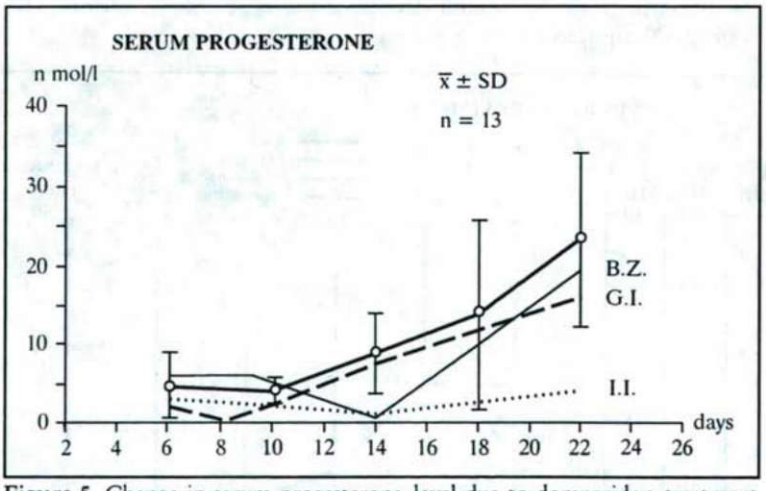

Figure 5. Change in serum progesterone level due to domperidon treatment from day 6 until day 22 of the cycle. (n=3). (Before TRI-REGOL treatment x +/-SD, n=13)

There was a lack of LH-peakat on the 14th day, however in 2 patients its concentration by the 22nd day exceeded the levels detected before treatment (Fig. 4.).

The 17-beta-oestradiol level markedly decreased and did not show typical „double-peaked" physiological curve (Fig. 5.).

Serum progesteron level remained within the physiological range both in the follicular and luteal period (Fig. 6.)

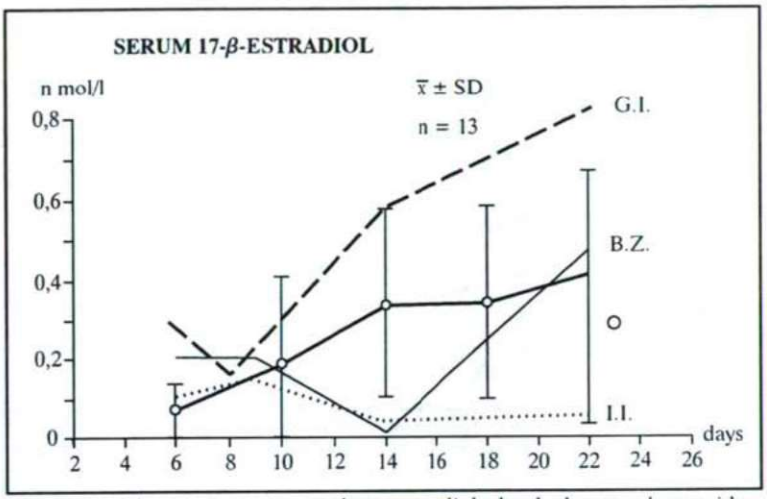

Figure 6. Change in serum 17-beta-oestradiol level due to domperidon treatment from day 6 until day 22 of the cycle. (n=3). (Before TRI-REGOL treatment x +/-SD, n=13)

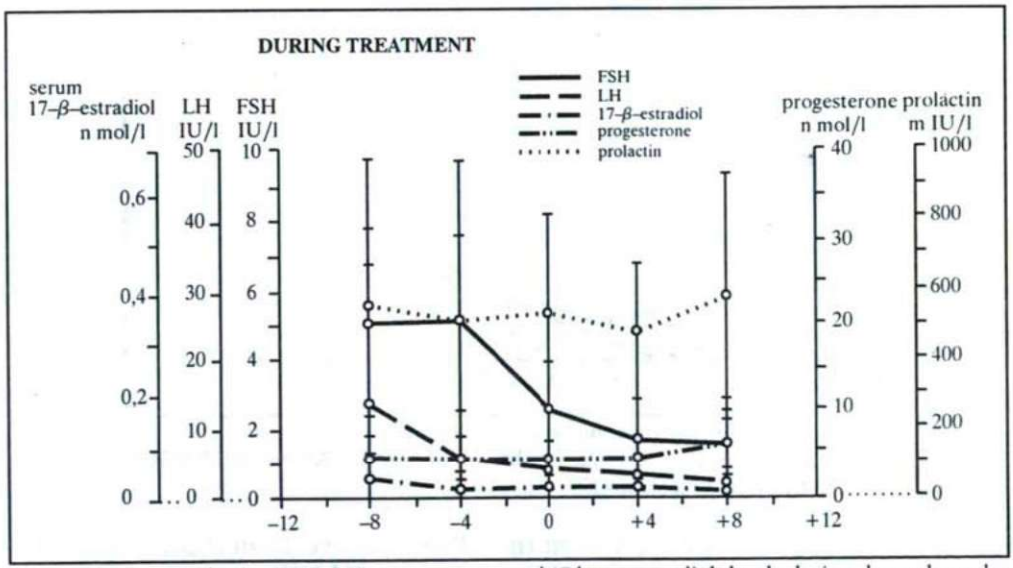

Figure 7. Serum prolactin, FSH, LH, progesterone and 17-beta-oestradiol levels during the cycle under TRI-REGOL treatment (x+/- SD, n=13).

Serum prolactin, FSH, LH, progesterone and 17-beta-oestradiol levels during the cycle under TRI-REGOL treatment (x +/-SD, n=13) (Fig. 7.).

The basic temperature curve of the patients was monophasic, typical of unovulatory cycles. Pregnancy occured in none of the cases and the menstruation cycle was restored.

Discussion

Female hypothalamic-hypophyseal regulation occurs at two levels. The lower level is regulated by the releasing factors and is known in literature as a tonic mechanism (FLERKO, 1962). Neural cyclic regulation is considered as an upper level. Szontágh (1976) found that lower progesteron levels affect the cyclic mechanism by inhibiting the releasing factor thus leading to a transient infertility.

Prolactin release is inhibited by hypothalamic dopamine. Some literary data indicate that prolactin regulates its release not only at the hypothalamic, but also at the hypohyseal level. That means that prolactin inhibits the hormon secreting activitiy of the hypophyseal lactotropic cells (HERBERT et al., 1979).

Domperidon as a dopamin inhibitor enhances prolactin secretion. The serum prolactin values become higher at the luteal phase of the ovulation cycle, compared to the follicular stage. Treatment with biphasic contraceptive tablets (Anteovin) fails to show such difference between the stages of the cycle, however the mean prolactin values were significantly lower, than in control cycles (FARKAS, 1988; FARKAS et al., 1986). Sas (SAS and FARKAS, 1979) showed that the luteal progesteron release is markedly lower, if plasma prolactin level is inhibited by bromocryptin to the values below 3 ng/ml (1 ng/ml = 32.5 IU/l). It seems that under physiological conditions prolactin plays no important role in ovulation. However if prolactin concentration is increased by domperidon or lowered below a certain level by bromocryptin, anovulation occurs. A further increase of concentration of the active substance not elevate the prolactin level. The effect will quickly reverse after cessation of treatment, indicating the reversibility of the increased prolactin level (FARKAS et al., 1989).

Treatment started from the 6th day of the cycle with 30 mg/day domperidon would abolish the LH peak typical of the ovulation. Pregnancy did not occur. Our patients had regular menstruation in regular time.

Despite the fact that our study has been performed only on 3 patients, we could clearly indicate that domperidon treatment elevates the serum prolactin level, abolishes the LH-peak and induces anovulatory menstruation. To clarify the anovulatory mechanisms elicited by dopamin inhibitor, further collection of data, wider clinical and laboratory studies are necessary.

Our data indicate that in women of reproductive age treated for gastrointestinal complaints with domperidon, oral anticonception becomes unnecessary. We expect that introduction of dopamine antagonists instead of hormonal anticonceptives woul overcome the pathological side-effects elicited by the latter on the reproductive system and the whole organism. Other dopamine antagonists substances could possibly solve the problem of postcoital anticonception, substituting the high doses of progesteron. Our results in this field will be published later.

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SHORT COMMUNICATION

PALAEOEPIDEMIOLOGY OF TUBERCULOSIS IN HUNGARY: PRELIMINARY RESULTS

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The purpose of the present study is to review the presence of osseous tuberculosis as a specific infectious disease in past human populations in Hungary. More than five thousand dry skeletons (n=5,848) have been examined from this point of view, all of which come from the Great Hungarian Plain and date from the time period of the 7–17th centuries (collections of the Department of Anthropology, József Attila University, Szeged). Our results are summarized in the Tables 1 and 2.

We have differentiated four chronological groups: the so-called „Avar Age" (7–8th centuries); the „Hungarian Conquest Period" (10th century); the „Árpádian Age" (11–13th centuries); and the so-called Hungarian „Late Middle Ages" (14–17th centuries).

We have diagnosed skeletal tuberculosis in 27 cases, several of which have already been published (see the list of references). Our results reveal that tuberculosis was present in Hungary almost all through the Middle Ages. Skeletal tuberculosis seems to have been more widespread in the 7–8th and the 14–17th centuries, while it is less frequent in the Arpadian Age. It has to be mentioned that Avar Age cemetery series without cases of skeletal tuberculosis are very rare. We have found 14 cases among the 1,988 examined specimens from this period. The living conditions especially in the Late Avar Period (8th century) — large agricultural settlements, animal breeding, rural life-style, high density of population, poverty (proved by archaeological data) — must have contributed to the spread of tuberculosis.

The absence of any signs of TB in the material from the period of the Hungarian Conquest has to be emphasized. Although our Hungarian ancestors kept animals (including cattles), their life-style was different from the late Avar sedentary life-style (8th century). The cases of skeletal tuberculosis have been found to be more frequent in the subsequent centuries following that the ancient Hungarians accompanied by other peoples settled down in the Carpathian Basin. We have concluded that in addition to other conditions (immunological, microbiological, etc.), the change towards sedentary life-style and the consequent increase in the density of human and domestic animal populations could certainly result in the increase of the disease frequency in the later part of the medieval period.

In accordance with the literature's data, our research has revealed that tuberculous alterations of spinal remains appear in the highest number in all of the examined periods. However, there are some differences between the series from the early medieval period (in sensu lato, our first three chronological groups) and the Late Middle Ages in the morphology and skeletal pattern of the lesions. Skeletal tuberculosis is not associated with rib lesions in the early medieval material. The revealed cases of osteo-articular tuberculosis dating from the Avar Age or the Arpadian Age show the typical characteristics of advanced-stage „healed" alterations (Figs. 1 and 2).

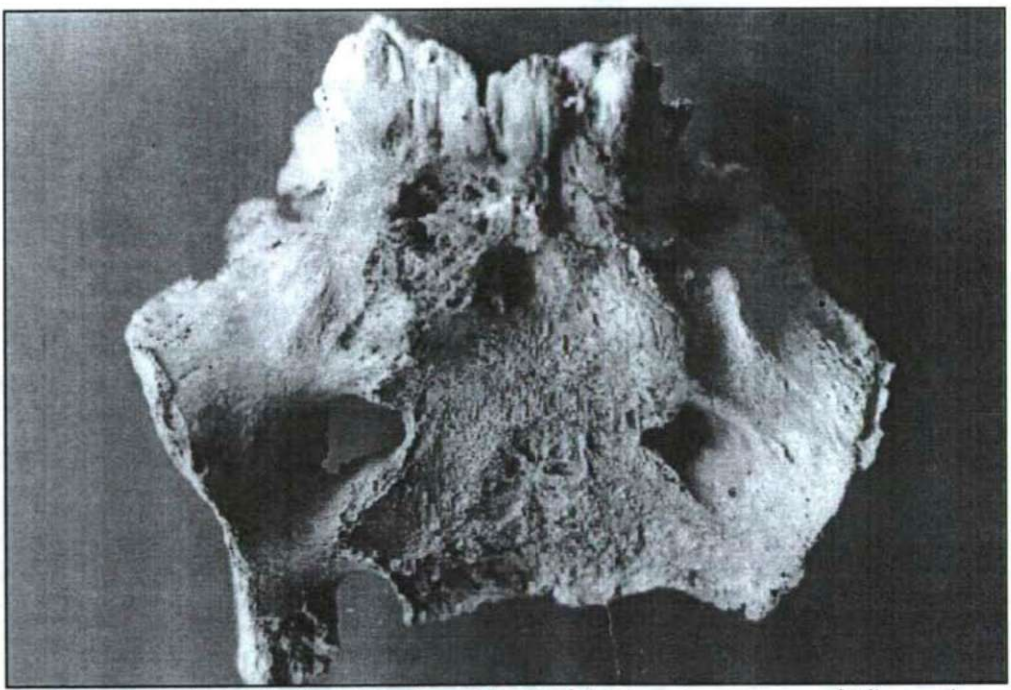

Figure 1. Lumbo-sacral tuberculosis (grave No. 90 of the Avar Age cemetery of Bélmegyer).

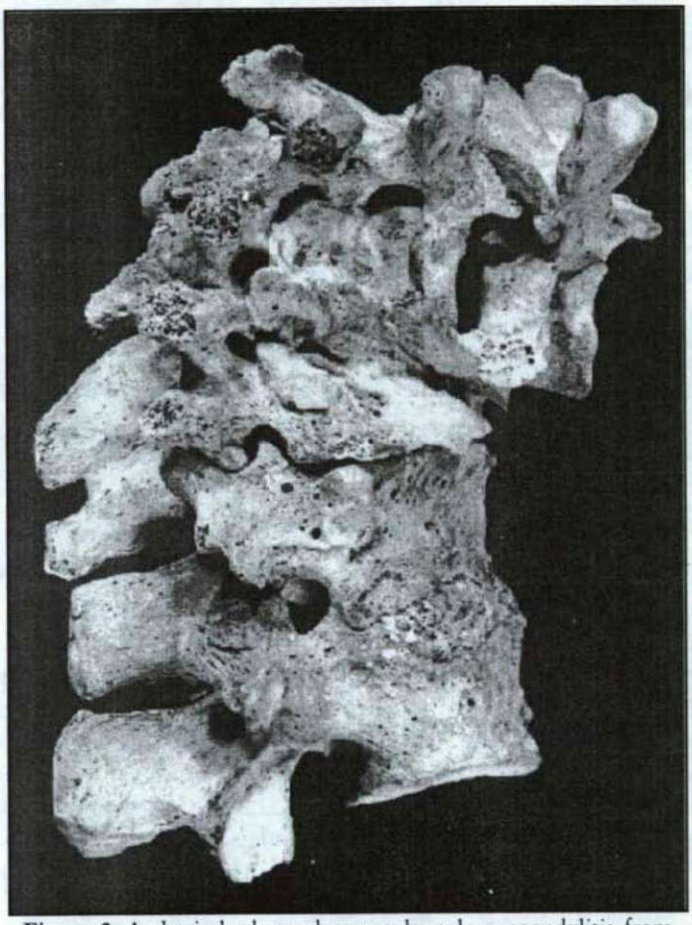

Figure 2. A classical advanced-stage tuberculous spondylitis from the Csongrád-Ellés cemetery (11-13th centuries, grave No. 183).

Although the commonest location of tuberculous alterations is still the vertebral column in the late medieval series, the revealed spinal lesions were caused by more active-stage spondylitis (with less signs of healing) and several cases of rib lesions has also been found. We have to mention an outstanding 17th century series (Bácsalmás, n = 173) in which 6 probable cases of osteotuberculosis can be observed. In the case from the Grave no. 61, the rib lesions (Fig. 3), just like similar alterations on seven other ribs on the same side, refer to tuberculous pulmonary infection and its probable direct spread through the pleura to the bones. We have to mention that the only one previously published palaeopathological case of TB-associated rib lesions from Hungary is also from the 17th century (ÉRY, 1982). Signs of rib lesions with infectious origin in two other skeletons in the series of Bácsal-

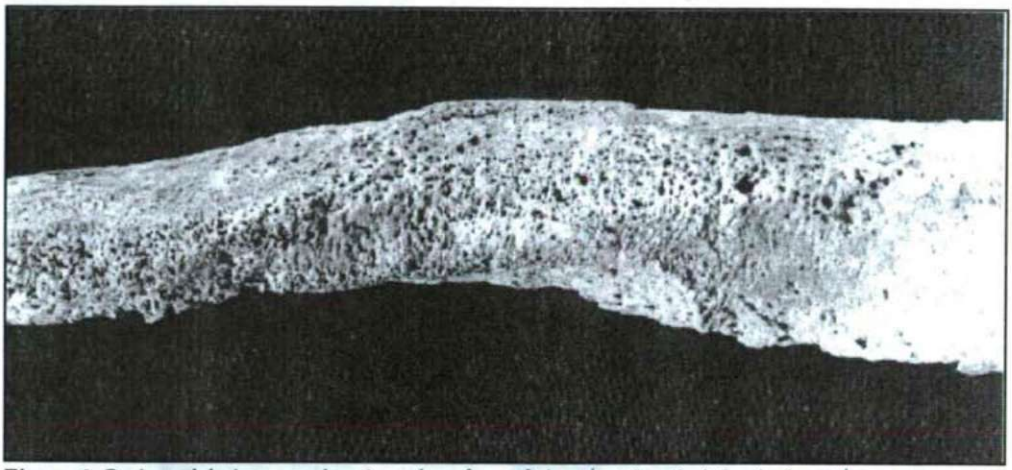

Figure 3. Periosteal lesions on the visceral surface of the 9th right rib (Bácsalmás, 17th century, grave No. 61), indicating tuberculous pulmonary infection and its probable direct spread to the bones.

más, the revealed active-stage („non-healed") vertebral lesions and the relatively high number of the affected skeletons suggest the different virulency of TB in this population compared to series from the Early Middle Ages. Differences in the way of transmission of the disease — for example the fact that pulmonary tuberculosis became more frequent in the populations from the Late Middle Ages — need also to be taken into consideration.

As it is well known in the paleopathological or epidemiological literature, the evolutionary model of TB is controversial (KELLEY, 1989). Tuberculosis presents several complexities to medical historians and paleopathologists because of the biological evolution of the bacteria and, on the other hand, the immunodeficiency, social disruption, and other variables aggravate its incidence. These facts stress the need for an interdisciplinary collaboration among paleopathologists, medical historians, epidemiologists, immunologists and microbiologists, to have a more available paleoepidemiological knowledge on the origin and evolution of human tuberculosis.

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Table 1: Evidences of osseous tuberculosis in past human populations in Hungary (Avar Ages: 7-8th centuries; Hungarian Conquest Period: 10th century)

Archaeological periode and site	Number of skeletons	No. grave	Type of tuberculosis	References
Avar Age: 7-8th centuries				
Bélmegyer	239	65	spinal	Pálfi, 1991
		90	spinal + hip	Pálfi et al, 1992
		215	knee (?)	Pálfi and Csernus, 1990
Szeged-Makkoserdő	152	209	spinal	Marcsik and Pálfi, 1992
		307	spinal	Marcsik, 1983
Csólyospálos	244	17	spinal (?)	Molnár and Marcsik (in press)
Székkutas	518	343	spinal	Pálfi, 1989
		385	spinal (?)	" "
		531	spinal (?)	" "
Pitvaros	209	12	spinal + hip	Molnár et al., 1998
Hetényegyháza	263	156	spinal	Szoboszlai, 1996
Sükösd	363	19	spinal + hip	Marcsik et al., 1997
		208	spinal	Jancsó, 1996
		218	spinal	" "
Avar Age total	1988	14 cases		
10th century				
Sárrétudvari	263	—	—	Pálfi, 1993
Püspökladány	230	—	—	Pauditz, 1995
Sándorfalva	104	—	—	Just, 1988
Algyő	77	—	—	Marcsik and Szalai (in press)
Szegvár-Oromdűlő	93	—	—	Marcsik, 1997
Szeged-Csongrádi út	11	—	—	Maczel, 1998
10th century total	778	—		

Table 2: Evidences of osseous tuberculosis in past human populations in Hungary (Árpádian Age: 11–13th centuries; Hungarian Late Middle Ages: 14–17th centuries)

Archaeological periode and site	Number of skeletons	No. grave	Type of tuberculosis	References
11–13th centuries				
Szegvár-Oromdűlő	259	275	spinal (?)	Marcsik, 1997
Szatymaz	286	—	—	Molnár et al., 1996
Kardoskút	160	—	—	Marcsik (under elaboration)
Püspökladány	371	383	spinal	Pauditz, 1995
Bácsalmás-Óalmás	54	—	—	Bozó, 1994
Bátmonostor	85	9	spinal (?)	" "
Csongrád-Felgyő	38	1	hip	Marcsik and Pálfi, 1993
Kecskemét-Gerőmajor	65	—	—	Ungvári, 1998
Hajdúdorog	612	434	spinal	Újvárosi, 1994
11–13th centuries total	1930	5 cases		
(14–17th centuries)				
Békéscsaba-környék	223	—	—	Farkas et al., 1991
Baja-Pető	209	—	—	Bozó, 1994
Kunfehértó	65	—	—	" "
Nagylak	45	—	—	" "
Röszke	67	—	—	" "
Gerla-Monostor	47	32	spinal	Farkas et al., 1991
Kecskemét-Ferences	323	125	hip (?)	Bozó, 1994
Bácsalmás-Homokbánya	173	39	spinal	Molnár and Pálfi, 1994
(17th century)		61	thoracic cage	" "
		85	thoracic cage	" "
		115	(?)	Széplaki, 1998
		142	spinal	" "
		160	spinal rib (?)	" "
14–17th centuries total	1152	8 cases		

ENVIRONMENTAL BIOTECHNOLOGY RESEARCH IN THE „UNIVERSITAS BIOTECHNOLOGY LABORATORY"

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The Department of Biotechnology of A. József University of Szeged, the Biological Research Center of the Hungarian Academy of Sciences, the Institute of Biotechnology of the Bay Z. Foundation for Applied Research, and the Food Technology Faculty of the Horticultural University agreed in 1996 to co-ordinate and combine forces and expertise related to the education, research, and development of biotechnology. The form of this collaboration is the „Universitas Biotechnology Laboratory", an institution without walls based on the mutual interest of the parties. Some of the joint projects are summarized as follows. Detailed presentations will follow in subsequent issues.

I. Biohydrogen

A fundamental and principal difficulty of the energy industry is that the formation of fossil fuels is much slower than the rate of their exploitation. Therefore the reserves which can be recovered in an energetically feasible manner

are shrinking parallel with an increasing world-wide energy demand. Among the alternative energy carriers, hydrogen is preferred because it is easy to transport and store and it burns to environmentally friendly water vapour when utilized. Hydrogen can be produced in biological systems: solar energy captured by the photosynthetic apparatus is converted into chemical energy through water splitting, the reaction forms oxygen and can also produce hydrogen. Upon utilization, these components are combined to form water and energy is released in a cycle driven by the practically unlimited and safe energy source of the Sun (SASIKALA et al., 1993).

In addition to offering an alternative for the global energy crisis, biologically produced hydrogen may also serve as reductant for numerous microbiological activities of environmental significance. Reductants are needed to convert CO_2, atmospheric nitrogen, nitrate, or sulfate into useful and/or environmentally safe products. For example, biogas is generated from industrial, agricultural, or household waste, nitrogen is fixed by plants which decreases their need for fertilizers, and nitrate is reduced to nitrogen in drinking water thereby eliminating a public health hazard common in both developed and less developed countries.

II. Environmental biotechnology

Reductants are required in many environmental applications because most compounds, generated as waste or hazardous material, are oxidized derivatives due to the presence of an oxidizing atmosphere on Earth. These compounds can be eliminated and recycled into the global C, N, O, or S natural cycles through reduction. Biological regeneration, bioremediation, utilizes reductants generated by biological systems, i. e., hydrogen produced *in situ* (KOVÁCS et al., 1996).

II. 1. Biogas

This principle has been employed in biotechnological control and management of the complex microbiological series of events that leads to biogas formation. We have shown that anaerobic biodegradation of solid household waste, waste water sludge, or animal manure can be accelerated. Biogas formation is intensified through the microbiological manipulation of the intermediate hydrogen production steps according to our patented procedure. The method has been tested in field experiments at the landfill depository of the city of Szeged, processing about 200,000 m^3 of solid household waste yearly, and in the 2,500 m^3 fermenter of a waste water sludge treatment plant (Bácsvíz Rt., Kecskemét). The technology is also exploited in an EUREKA project of the European Union, involving Swedish, Polish, and Austrian partners.

II. 2. Denitrification

Biological hydrogen metabolism is utilized in another approach to eliminate nitrate from drinking water. It is well known that nitrate is a hazardous material endangering human health and life. Nitrate is most commonly taken up through contaminated drinking water. Nitrate accumulates in natural water reserves mainly because of the improper application of fertilizers in agriculture and because of inadequate waste water treatment (a local example of human negligence is the lack of a municipal waste water treatment facility in our home base city of Szeged). Nitrate is the most oxidized form of nitrogen and can be returned into the natural nitrogen cycle after reduction to the completely innocent nitrogen gas.

The biological denitrifying process developed at the UBL-Szeged consists of a mixed bacterial population, which is capable of reducing nitrate to nitrogen in an effective and economic way using the *in situ* produced hydrogen for reduction. Scale up work is being done in collaboration with Nitrokémia Chemical Works, Inc., a major chemical plant in Hungary producing excellent nitrate selective ion-exchange resins. The process is the subject of an other EUREKA projects at the European Union level, where the Hungarian team collaborates with Czech, Dutch, French, and German partners.

III. Hydrogenase

The understanding of molecular fundamentals of hydrogen production and utilization among microbes is a goal of supreme importance both for basic and applied research applications. The key enzyme in biological hydrogen metabolism is hydrogenase, which catalyses the formation or decomposition of the simplest molecule occurring in biology: hydrogen.

$$H_2 \Leftrightarrow 2H^+ + 2e^-$$

The simple-looking task is solved by a sophisticated molecular mechanism. The majority of hydrogenases are metalloenzymes, harbouring Ni and Fe atoms. Like most metalloenzymes, hydrogenases are extremely sensitive to inactivation by oxygen, high temperature and other environmental factors. These properties are not favourable for several potential biotechnological applications.

In metal containing biological catalysts it is the protein matrix surrounding the metal centers, which provides the unique environment for the Fe and Ni atoms which allows hydrogenases to function properly, selectively, and effectively. Therefore, the main goal of our basic research is to understand the protein-metal interaction.

The problem is not simple to address as some of the methods for scientific investigation provide information on the metal atoms themselves without directly observing the protein matrix around them. Other modern techniques at our disposal reveal details of the protein core, but do not expose the metal centres within. A combination of these two approaches, i. e., molecular biology and biophysics, is expected to uncover the fine molecular details of the catalytic action of metalloenzymes.

An important condition of successful research is the choice of the target microorganism best suited for scientific investigation. Several years ago we selected the hydrogenase enzyme(s) of photosynthetic bacteria. One of the purple photosynthetic bacteria *(Thiocapsa roseopersicina)* contains a hydrogenase, which displays outstanding stability among enzymes possessing the same catalytic function (KOVÁCS et al., 1996, RÁKHELY et al., 1998). Heat stability does not provide an advantage for the bacterium in its natural, cold water marine environment. Understanding, through molecular biological studies, the molecular factors that stabilize this hydrogenase (COLBEAU et al., 1994) is expected to help design various enzymes equipped with resistance to the inactivating effect of high temperature, oxygen, and/or proteolytic attack for future biotechnological use.

In the European Union and Associated States, research networks are formed around topics of outstanding economic and/or scientific interest. One of these networks is COST Action 8.18 „Hydrogenases and their Biotechnological Applications". COST Action 8.18 includes practically every laboratory of significant contribution to hydrogenase research, i. e., 45 laboratories from 13 European nations. The network has been co-ordinated by the head of the Szeged laboratory for the third consecutive year.

IV. Hyperthermophiles

Hyperthermophilic microorganisms grow above 80°C, and cannot multiply below 70°C. Most hyperthermophiles are archaea. Hyperthermophiles occur in various habitats, e. g., deep sea volcanic sites, hot water spring and even chimneys. By definition, enzymes operating in these unusual creatures are heat stable, therefore they offer an obvious advantage for biotechnological applications. Hyperthermophilic archaea also represent the oldest form of life on Earth. In the chemolithotroph metabolism, characteristic of hyperthermophilic archaea, hydrogen metabolism plays a crucial role. Due to the considerable technical difficulties in cultivating these microorganisms in the laboratory, only a few of the hyperthermophilic hydrogenases have been purified and characterized. We have developed a novel technique to plate hyperthermophilic archaea (RÁKHELY and KOVÁCS, 1996), this is the first major step to study their microbiology, molecular biology

and genetics. This information is needed for the multiple biotechnological exploitation of hyperthermophiles.

V. Methanotrophs

Methanotrophic bacteria typically contain another metalloenzyme, methane monooxygenase (MMO). Methanotrophs utilize methane as their sole source of carbon and energy. Methane is oxidized to CO_2 through methanol, formaldehyde and formate intermediaries. The first and most important step in this sequence of reactions is the methanol conversion, catalysed by MMO.

MMO can also attack several compounds representing serious environmental and public health hazard, such as chlorinated hydrocarbons.

The solution to the molecular puzzle of MMO activity is in the intimate relationship between the protein matrix and the metal centres embedded in it, as in the case of hydrogenases.

A clear view of the methane and hydrogen metabolism in methanotrophs will be significant for advancing molecular enzymology as well as for practical utilization of methanotrophs to produce alternative energy sources and environmental protection, improving the general quality of life.

In order to fully exploit the benefits of methanotrophic biotechnology, strains that thrive at elevated temperatures are needed. Heat tolerant methanotrophs have not previously been known. We have isolated methanotrophs which grow at 55–60°C [6] and described a new species, *Methylocaldum szegediense* OR2, that grows up to 72°C (BODROSSY et al., 1997). Characterization of heat stable MMO and hydrogenase enzymes from these novel methanotrophs will lead to the development of a new generation of biocatalysts for practical use.

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THIRTY YEARS OF THE DEPARTMENT OF COMPARATIVE PHYSIOLOGY

In 1966 the leadership of the József Attila University decided to develop and modernize the biological education and research activity at the Faculty of Natural Sciences. Within the frame of modernization some of new departments have been founded, among them the Department of Animal Physiology (or by its present name: Comparative Physiology). Animal physiology had been taught before at the Department of General Zoology and Biology (headed by Academician AMBRUS ÁBRAHÁM) by FERENC BICZÓK Ph.D., among other zoological disciplines, without separate teaching and research staff. After the retirement of Professor ÁBRAHÁM the anatomy, histology and embryology staff moved to the Department of Systematic Zoology (headed by Academician GÁBOR KOLOZSVÁRY). At the same time a new department: Department of Animal Physiology was founded.

According to a decree of the Ministry of Cultural and Education Affairs, the Department of Comparative Physiology started its activity officially on 1st of November 1967. OTTÓ FEHÉR M.D. was invited to be the head of the new department. OTTÓ FEHÉR was previously a university lecturer at the Department of Physiology of the Medical School in Debrecen. He started to develop a real physiological deparment immediately. The laboratories had to be reconstructed for the purpose of physiological work, a room for laboratory animals had to be built, and the department needed urgently modern physiological sets for teaching and reserch. With the financial support of the Ministry of Education Affairs and of the University, the Department furnished the first physiological laboratory. In starting the teaching and research activity one of the founders of the department, LAJOS ERDÉLYI played an outstanding role. In the organizational and technical work GÉZA TURY helped much to head of the department.

To the group of the founders (OTTÓ FEHÉR, LAJOS ERDÉLYI and GÉZA TURY) later joined: GÁBOR BÁLINT M.D., MAGDOLNA SZENTE, ISTVÁN PÓR, IMRE GAÁL, ATTILA BARANYI, JÓZSEF TOLDI, HORST SCHULZ, RÓBERT NOVÁK, MÁRIA FALUDY, FERENC LÁSZLÓ and recently CSABA VARGA. (See the actual staff later).

At the beginning the Electronmicroscope Laboratory functioned as a part of the Depertment, headed initially by Prof. BERTALAN CSILLIK M.D. and later by FERENC JOÓ M.D. The scientific staff of this laboratory consisted of NORBERT HALÁSZ and ÁRPÁD PÁRDUTZ. The laboratory was supported by the Hungarian Academy of Sciences. Since 1972 the Laboratory has continued its work as the

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Group of Molecular Neurobiology in the Biological Research Center of the Academy in Szeged.

In 1973 a new Electron Microscopic Laboratory was established to aid the scientific research of the Biological Departments with the leadership of IMRE ROJIK and with the technical assitance of Mrs. ZSUZSA FEJES.

Until the establishment of the two other new departments: Dept. of Biochemistry and Dept. of Genetics (established in 1974 and 1980), the Department of Animal Physiology gave place for the Group of Genetics (leader was LAJOS ALFÖLDI M.D.) and the Group of Biochemistry (leader was BÉLA MATKOVICS M.D.).

From the beginning until 1977 the Department gave place and other facilities for the work of the biologist-didactician LÁSZLÓ KÖRTVÉLYESSY Ph.D., who until his retirement in 1977 carried out succesful educational and scientific activity.

The range of the educational tasks covered by the Department was rather wide already. During the first two decades of the Department's history, students who became teacher of biology and chemistry and students in biology (were alltogether about 40 students) attended lectures and practical courses in comparative physiology in the Department. Lectures in school-hygiene and animal husbandary were also delivered by members of the Department. Nowadays however, more than 110 students (who become teachers of biology, researchers in biology, psychologists) attend lectures and practical courses in comparative physiology, molecular physiology, neurophysiology and neuropharmacology, neuroplasticity, psychophysiology.

Training in comparative physiology at the university level started in the ELTE (Budapest) in 1965 (Prof. GYÖRGY ÁDÁM M.D.) then in the Department of Animal Physiology (since 1974 Comparative Physiology) at the JATE (Prof. OTTÓ FEHÉR M.D.). As a first step, a guide book was published by OTTÓ FEHÉR, LAJOS ERDÉLYI, JÓZSEF FAISZT, GÁBOR HOLLÓSI and MIHÁLY KURCZ, with the title „Exercises and Experimental Demonstrations in Comparative Physiology". In 1975 Academician GYÖRGY ÁDÁM and Prof. OTTÓ FEHÉR published the Textbook „Comparative Physiology". The book was rewarded with a „Niveau Prize" by the Ministry of Education in 1977. Later, this book was edited and published several times, recently titled as „Physiology for Biologists".

The Department was the first in Hungary where the teaching of molecular physiology was introduced in the late eighties. The staff of the Department wrote a book entitled: „Molecular Basis of Physiological Processes" (edited by O. FEHÉR). This book was followed by a Compendium: „Voltage- and Ligand Activated Ion Channels" written by LAJOS ERDÉLYI in 1996. Recently published book of the Department is the „Development and plasticity of the mammalian nervous system" written by MAGDOLNA SZENTE and JÓZSEF TOLDI.

During the last 30 years the infrastructure (instrumentation) and in relation with it, the practical programs in the student laboratory has been basically renewed three times: at the beginning, mechanical kymographs (SÁNDOR MOTZWICKLER) served for registration of physiological phenomena, then in the middle eighties, the

common production of the mechanical and electrical workshops (FERENC DOBÓ and FERENC GYULAI) amplifiers, stimulators, transducers and oscilloscopes served for the student laboratory exercises. Very recently (in 1997) student laboratory infrastructure has been completely changed, the students work on full-computerised equipments.

In 1994 the postgraduate (doctoral) program of the Department (entitled: Neurobiology) has been accredited. Nowadays, it is a common program of two departments (Dept. of Comparative Physiology and Dept. of Zoology and Cell Biology). As the first postgraduate student of this doctoral program, GÁBOR TAMÁS got his Ph.D. degree in 1996.

Thus comparative physiology during the last 30 years has gained its proper place in our University as an important discipline in the education and scientific research.

The main objective of the scientific research of our Department is the nervous system. Though the members of the Department changed a lot during the last 30 years, the Department has developed and it is one of the well known institutes of neuronal sciences in Hungary. The main directions of the research are as followes:

1) The mechanism of epileptogenic phenomena in the central nervous system (MAGDOLNA SZENTE).

2) Injury induced neuronal plasticity in peripheral and central nervous system (JÓZSEF TOLDI, ZSOLT KIS and TAMÁS FARKAS).

3) Pharmacological and ionic modulation of membrane and postsynaptic currents in the nervous system of Mollusks (LAJOS ERDÉLYI).

4) Physiological effects of vasopressin analogues (FERENC LÁSZLÓ and CSABA VARGA).

5) Synaptic and autaptic control of excitability by GABAergic neurones in identified cortical networks (GÁBOR TAMÁS).

The repertoire of scientific research methods applied by the Department has become rather wide and is of interdisciplinary character. The most important methods are as followes: stimulation and recording in any part of the central and peripheral nervous system with macroelectrodes, electroencephalography, stereotaxic techniques, intracellular recording and stimulation, labelling and filling of neurones, polarization and conductance measurement, the voltage and patch clamp methodes, extracellular microelectrode techniques, microiontophoresis, standard methods for examination of heart, blood circulation, gel chromatography and gel electrophoresis, HPLC, ELISA, standard electronmicroscopic methods, light- and electronmicroscopic autoradiography, etc.

The members of the staff have participated in several national and international congresses and symposia, published more than 200 full-length articles. Most of the articles have been published in international papers with valuable impact, including e. g. the Nature.

The staff of the Department has a widespread collaboration both in Hungary (SZBK, SZOTE, SOTE, ELTE) and in abroad (USA, Oxford, Paris, Göttingen, Tokio).

In 1996, five out of the seven staff members had the degree Doctor of Biological or Medical Science and two of co-workers had the degree Candidate of Biological Science. Since that time OTTÓ FEHÉR and FERENC LÁSZLÓ retired, and ATTILA BARANYI left the University.

The members of the Department were/are as followes from the beginning:

The heads of the Department: OTTÓ FEHÉR M.D., D.Sc. (1967–1988), LAJOS ERDÉLYI C.Sc. (1988–1994), ATTILA BARANYI D.Sc. (1994–1996), JÓZSEF TOLDI D.Sc. (1996–).

The members of the teaching and reserach staff who have already left the Department: GÉZA TURY Ph.D. (1967–1977), GÁBOR BÁLINT M.D., Ph.D. (1969–1970), ISTVÁN PÓR Ph.D. (1971–1975), LÁSZLÓ KÖRTVÉLYESSY Ph.D. (1967–1977), IMRE GAÁL Ph.D. (1973–1978), ROBERT NOVAK, FERENC PONGRÁCZ (1975–1983), HORST SCHULZ (1981–1988), OTTÓ FEHÉR M.D., D.Sc. (1967–1996), FERENC LÁSZLÓ M.D., D.Sc. (1988–1996).

The members of technical staff who have already lef the Department:

Secretaries: Mrs. ANNA BASCH, Mrs. GABRIALLE VÖRÖS, Mrs. MAGDOLNA FUTO, Mrs. JUDIT MADURKA, Mrs. ERZSÉBET DOBÓ, Mrs. MAGDOLNA OSZTROLUCZKY.

Assistants: Mr. GÉZA MRÁZ, Ms. ERZSÉBET DÁNOS, Ms. ANIKÓ VÁGVÖLGYI, Mrs. ANIKÓ SZŰCS, Mrs. ÁGNES VECSERNYÉS, Mrs. ZSUZSA FEJES, Ms. GABRIELLA PAP.

Mechanics at the workshop: SÁNDOR MOTZWICKLER, PÉTER TRÁM, FERENC SZŰCS, FERENC DOBÓ.

The active members of the Department:

Head: JÓZSEF TOLDI D.Sc.

Co-professor: MAGDOLNA SZENTE D.Sc.

Assistant professors: LAJOS ERDÉLYI C.Sc.

Assistant lecturer: CSABA VARGA (Ph.D.)

Post doc.: (on ZOLTÁN MAGYARY fellowship): GÁBOR TAMÁS Ph.D., (on post. doc. fellowship of MKM) TAMÁS FARKAS (Ph.D.).

Senior researher: IMRE ROJIK C.Sc.

Research fellow: Mr. ZSOLT KIS

Electric engineer: Mr. FERENC GYULAI

Secretary: Mrs. MARGIT VEKETY

Assistants: Ms. ANIKÓ BERKÓ, Ms. GABRIELLA MÉSZÁROS, Mr. SÁNDOR HUSZTA.

PhD students: Ms. BARBARA BARNA, Ms. ÉVA MORSCHL, Mr. TAMÁS CSÓTI, Mr. Mrs. VERONIKA ZSIROS, ANDRÁS SZÁSZ, Mr. TAMÁS VIRÁG.

Dr. J. Toldi

SCIENTIFIC PROGRAM OF THE MEETING HELD ON OCCASION OF 30TH ANNIVERSARY OF THE DEPARTMENT OF COMPARATIVE PHYSIOLOGY OF J. A. UNIVERSITY

Opening

Dr. J. TOLDI: Welcome the celebrating audience

Welcome:

Prof. DR. R. MÉSZÁROS, Rector of the József Attila University.
Prof. Dr. GY. TELEGDI, Academician, President of the Academy Group at Szeged.
Dr. E. MIHALIK, head of the Council of Biological Departments.
Prof. Dr. P. HALÁSZ, head of the Neurology Clinic at OTE (Budapest).
Review: Prof. Dr. O. FEHÉR.

Lectures:

L. ERDÉLYI: Ionic and pharmacological regulation of the A-current in snail neurons.
I. ROJIK: Study of active neuron with the aid of microscope.
M. SZENTE: Generating of epileptic activity in the cerebral cortex.
Cs. VARGA: Study of effects of neurohypophyseal hormone antagonists.
Prof. Dr. J. R. WOLFF: Cortical plasticity: 19 years of co-operative research for underlying mechanisms.
T. FARKAS: Plasticity in the somatomotor system of the rat.
Á. PÁRDUTZ: The mechanism of transmitter release.
J. TOLDI: Compensatory mechanims after sensory deprivation.

PROF. DR. OTTÓ FEHÉR IS 70 YEARS OLD

OTTÓ FEHÉR was born in Debrecen in 1927. While still a medical student, he started his scientific career at the Department of Physiology headed at that time by Professor ISTVÁN WENT. He involved in a team which worked on the probem of regulation of heart and blood circulation. He received his M.D. in 1951 and started his postgraduate study in physiology of the nervous system. It is generally not known, that he was the first who proved that in the postsynaptic membrane of the ganglion cells there are two types of acetylcholine receptors: a nicotinic receptor type and a muscarinic one, differing in their physiological role and pharmacological responsiveness. That time he published it in German. Probably, that is the reason of relative unknown of his discovery though, it has been confirmed in the literature repeatedly. Already that time he had several students (SÁNDOR DAMJANOVICH, PÉTER HALÁSZ, FERENC MECHLER, ELEMÉR LÁBOS, TIBOR SZABÓ and GYULA MÓZSIK) who were active as young scientific investigators, and now they are well known personalities of Hungarian science.

He got his C.Sc. degree in 1960 for the work on the „The role of the acetylcholine-cholinesterase system in the ganglionic transmission of impulses". As a young and enthusisastic researcher, he was the first in Hungary, who introduced the recording of action potentials from peripheral nervous structures.

At the beginning of sixties his attention turned to the physiology of the central nervous system. In collaboration with PÉTER HALÁSZ, FERENC MECHLER he made relevant observations on the origin of cortical convulsive potentials and their relation to the sensory evoked potentials.

It was a mile stone in his career when in 1967 he was invited to Szeged to be head of Department of Zoophysiology being founded at the József Attila University, that time. From the beginning he made efforts to organize the training of students in comparative physiology and offer facilities for practical courses. In this work he was assisted by his first colleagues LAJOS ERDÉLYI and GÉZA TURY. That

was the time when he met with young morphologists: ÁRPÁD PÁRDUTZ, FERENC JOÓ and NORBERT HALÁSZ, and activated them to start together an interdisciplinary research to elucidate correlations berween electrophysiological signs of synaptic transmission and morphological changes in synaptic ultrastructure. In the early seventies he initiated a new trend of research on the fate of labelled amino-acids in the cerebral cortex and intorduced — together with IMRE ROJIK — a new method for visualising active nervous structures making use autoradiography.

OTTÓ FEHÉR got his D.Sc. in 1973 for the work on „The origin of cortical evoked and convulsive potentials".

In early seventies he invited young people to be his co-workers. MAGDOLNA SZENTE, ATTILA BARANYI and finally JÓZSEF TOLDI became the new members of the department. Together with ATTILA BARANYI, OTTÓ FEHÉR investigated the heterosynaptic facilitation which is considered to be one of the basic mechanisms underlying formation of memory traces. With MAGDOLNA SZENTE — and temporarily with FERENC PONGRÁCZ — he investigated the central epileptogenic phenomena. This common research brought him to a new scientific field: with the aid of a mathematician TIBOR GYIMÓTI he formulated a computer model of epileptic membrane. Since that time he is interested in formulating computer models (very recently with students: TAMÁS VIRÁG, RÓBERT SCHNELL). Together with JÓZSEF TOLDI he started to work on the scientific problem of neuronal plasticity. This common research lasted until his retirement.

In the eighties his research work with LAJOS ERDÉLYI and ANDRÁS PAPP on *Helix* neurons served as a basis for discovery of new anti-convulsive substances. These substances were tested pharmacologically and toxicologically by HORST SCHULZ, who was also his co-worker for about ten years. In the early eighties he invited FERENC GYULAI an electric engineer to the Department and with his help OTTÓ FEHÉR introduced new techniques and devices into the educational and scientific activity of the Department.

Since 1978 OTTÓ FEHÉR participated in a common scientific research project together with the Institute of Anatomy at Georg August University, Göttingen (headed by professor J. R. WOLFF) and with the Neurobiological Group of the Biological Research Center (headed that time by FERENC JOÓ).

He had a fruitful technical collaboration with the Biological Institute of the former Soviet Academy (Pushchino) and with the Biological Research Institute of the Yugoslavian Academy (Beograd).

In 1977 OTTÓ FEHÉR was awarded the Academy Prize for his pioneer activity in introduction of new electrophysiological methods in Hungary. Together with professor GYÖRGY ÁDÁM he is co-author of the university textbooks Comparative Physiology and Physiology for Biologists. He is also co-author and editor of the practice book of this discipline.

In the late eighties he initiated the teaching of molecular physiology in the course of education of biologists. He was the editor and one of co-authors of the book of „Molecular Basis of Physiological Phenomena", published first in Hungary.

He was active member of several scientific committees and societies. He organized several congresses and international symposiums. Three of his co-workers have been awarded the degree Doctor of Biological Science and another two of his co-workers the degree Candidate of Biological Science. In collaboration he has published more than 105 scientific artickles.

The Department of Comparative Physiology at the József Attila University was founded 30 years ago by professzor FEHÉR. He gave a characteristic educational and scientific profile for his neurobiological school and brought it in many respects to international level. His progressive attitude to scientific issues which is still there and working in each day in his disciples, in the staff of the Department, and that gives the hope in the further successes.

Professor FEHÉR retired in 1996. As a retired professor he is still active, giving lectures, formulating new computer modells e.g. of central nervous structurs.

We all wish professor OTTÓ FEHÉR good health and further successful activity.

Dr. J. Toldi

PROF. DR. FERENC ZSOLDOS IS 70 YEARS OLD

FERENC ZSOLDOS Professor Emeritus of József Attila University is 70 years old. This anniversary offers the occasion to recall his course of life, the milestones of the half century of his scientific career.

FERENC ZSOLDOS was born in Sarkad, Hungary, on 24th March, 1927. After secondary school studies in Sarkad and then Békéscsaba, he matriculated in 1947. He next enrolled as a student of biology at the Faculty of Natural Sciences of Eötvös Loránd University, in Budapest, from which he received his diploma in 1952. Following this, he took part in postgraduate training for three years at the Department of Plant Physiology, and then from 1955 worked as an assistant lecturer at the Department of Applied Botany of the University. He moved to the Department of Plant Physiology of the University in Szeged in 1957. He held the position of research worker till 1974, in which year he was appointed Reader, followed in 1984 by his appointment as Professor. He became Chairman of the Department of Plant Physiology in 1985, and continued to hold this position until 1995, and retired in 1996.

FERENC ZSOLDOS started his educational and teaching activities at Eötvös Loránd University where, while still a university student, he regularly took part as a demonstrator in the botany practicals. Later, following his move to Szeged, he led plant physiology practicals, held courses of special lectures, and delivered lectures in the main courses on plant physiology. Under his guidance, an appreciable number of university students have prepared their diploma work and theses for their degrees, or have obtained their university doctoral degrees. He delivered regular lectures of plant physiology and courses of special lectures on the topics of mineral nutrition, deficiency diseases of plants, and the practical use of hydroponic cultures, within the framework of national (agricultural engineer, teacher, etc.) training courses and postgraduate training courses.

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FERENC ZSOLDOS started his research work in 1952 at the Department of Plant Physiology of Eötvös Loránd University, where in 1957 he prepared and successfully defended his university doctoral dissertation [*Studies on the nitrogen metabolism of rice*], and later his dissertation for the degree of Candidate [*Physiological studies in rice seedlings*] and Doctor of Science of the Hungarian Academy of Sciences [*Effects of environmental factors on ion uptake by plants*]. His scientific activities were greatly influenced by the circumstance that, first as a scholar, and then as a visiting scientist, he was able to spend a long period in 1962 at the Laboratory of the International Atomic Energy Agency near Vienna (Seibersdorf). Here he learned the modern isotope techniques relating to ion-transport research. Later he started dealing with the effects of environmental stress factors (low temperature, pH, nitrite, aluminium, etc.) and various, biologically active compounds (e.g. herbicides) on the nutrient uptakes and growth of grain crops. In connection with this topics, he has published 173 papers in national (Acta Biol. Hung., Acta Biol. Szeged., Bot. Comm., etc.) and international (Nature, Physiologia Plantarum, Plant and Soil, J. Plant Physiology, etc.) scientific periodicals as well as in the publications of international congresses.

FERENC ZSOLDOS is at present a member of several Hungarian (Hungarian Society of Biology, Hungarian Society for Plant Physiology, Plant Physiological and Botanical Committees of the Hungarian Academy of Sciences) and foreign (European Society for New Methods in Agriculture, Federation of European Societies of Plant Physiology) scientific societies and committees, respectively. He was editor of the journal Botanikai Közlemények (Botanical Communications) 1980–95, and an editorial board member of Acta Biologica Szegediensis (1984–), Physiologia Plantarum (1978–92) and Oryza (1985–90). He is a member of advisory board of the „Japan Prize" Selection Committee (1985–).

Besides his university educational and research activity, FERENC ZSOLDOS has been, or still is taking an active part as project leader in the direction and elaboration of objectives connected with plant mineral nutrition in collaboration with Hungarian (Institute of Biophysics, Biological Research Center of Hungarian Academy of Sciences, Szeged, ERDEI, L.; Cereal Research Institute, Szeged, BÓNA, L.; Research Institute for Irrigation; Rice Laboratory, SZARVAS, S. KISS, I.) and foreign (Institute of Agriculture, Austrian Research Center, Seibersdorf, Austria, HAUNOLD, E.; University of Bayreuth, Germany, KOMOR, E.; University of Udine, Italy, MACRI, F. and VIANELLO, A.) partners. The co-operation of these partners in a multidisciplinary research was emphasised, when FERENC ZSOLDOS and co-workers (PÉCSVÁRADI, A., TARI, I., SZABÓ, M., NAGY, M. and VASHEGYI, Á.) launched a project entitled „Study of Physiological Changes in Plants Exposed to Nitrite: an Environmental Stress Factor and/or N Source" in PHARE ACCORD Programme (1993–94) supported by the European Community.

As an acknowledgement of his successful activities in university education, research and scientific life, in 1987 he was awarded the honour „For Outstanding Work" by the Hungarian Cultural Minister, in 1992 an Award of Hungarian Academy of Sciences for the activity in the stress physiology of plants, and in 1996 a title of Professor Emeritus from the József Attila University.

Now he is an active Professor Emeritus, still working untiringly in teaching and research projects of the department, participates in committees of the University and the Hungarian Academy of Sciences, in scientific public life.

We wish professor FERENC ZSOLDOS good health and successful activities.

László Erdei

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AN OBITUARY TO PROF. DR. FERENC BICZÓK (1912–1998)

The biologists of Szeged are mourning again. Professor FERENC BICZÓK, retired emeritus Professor, died suddenly on 26 May, 1998. His colourful career was not very well known while he was alive, apart from his autobiography „My life", published formerly.

He was born in Szeged on 1 October, 1912. His father died in the First World War which made life very difficult for the family where there were 5 people. Although he was an excellent student both at the primary and secondary schools, he had financial difficulties and had to take on manual work to get by. It was not earlier than in 1929 that he could continue his studies at the Royal Catholic Teacher Training College in Szeged. He was good at drawing, so he carried on at the College of Fine Arts, but he did not feel comfortable there. After that he was dealing with music. He had taught biology and chemistry when he was a student, but after finishing his studies he carried on studying biology, chemistry, geography and music at the Department of Zoology of the Teacher Training College for Civil Schools. This made possible for him to find a job as a parish choirmaster in a village near Szeged.

He started his scientific career at the Department of Zoology and it was there that he delivered his first scientific lecture after being inspired by Professor AMBRUS ÁBRAHÁM.

After having finished the 4-year Teacher Training College, he went on studying at the Apponyi College. It was there that he received his degree entitling him to teach in secondary schools. It was also there that he obtained his doctoral degree. However, his inauguration took place only in 1941, and after that he got a job as a teacher at the Madách-street civil boys' school in Szeged.

Later on he went to work at the Teacher Training College in Ujvidék, but escaped to Germany in 1944 due to the war where he could make good use of his talent in music. Although he got an invitation to the University of Hannover, he came home to Hungary in November, 1945.

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In February, 1946 he got a job at the Teacher Training College in Pápa, and he worked there for the next 6 years. After having rejected several promising job offers, he accepted a lecturership at the Institute of General Zoology and Biology of the University of Szeged in 1953. He was working there under the leadership of Professor AMBRUS ÁBRAHÁM until 1974 when he retired. However, he carried on his research at the Clinical Laboratory of the Medical University of Szeged.

His scientific research activity, carried out at different places, reached a successful conclusion in Szeged. In 1959 he successfully defended his Candidate Thesis entitled „Investigation of soil-living Protozoa, with particular emphasis on rhyzosphere", and he was given a readership in 1960. He was always interested in unicellular organisms, he employed various methods for studying them. Although he was offered professorships at the Medical University of Szeged as well as at the University of Debrecen, but he stayed at the Department of Zoology.

Apart from scientific research, he also dealt with pedagogy in higher education and wrote several assays in this topic.

During his active life, however, he was frustrated several times which made his life embittered.

His achievements were recognized and appreciated by his contemporary scientists. He was the president of the Protozoological Society and a member of the Presidium of the Hungarian Biological Society. In addition, he was a member of the Association Internationale de la Science du Sol (since 1963), the Royal Society of Medicine (since 1965) and the Society of Protozoologists (since 1966). He received professorship with a delay, only in 1989. His bibliography will help us appreciate his scientific achievements.

Although he spent most of his life in Szeged, in 1990 he moved to a retired people's home in Budapest with his wife. It was there that he suddenly finished his fruitful life at the age of 86. He was buried in the Terézváros church of Budapest on 20 June 1998. Let him rest in peace!

Dr. Gyula L. Farkas

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Gy. L. Farkas

AN OBITUARY TO DR. GYŐZŐ CSONGOR (1915–1997)

Dr. GYŐZŐ CSONGOR was born in Szeged on 27 February. He received the first impressions about interest in nature from KÁLMÁN CZÓGLER, his teacher at the BAROSS GÁBOR Secondary School. He went to university in Szeged and Kolozsvár. Even as a student, he carried out investigations in Greece, Turkey, Switzerland, Austria and Germany. His botany teachers at the university were Professor GYÖRGY GYŐRFFY and forest engineer FERENC KISS. After graduation, he was an assistant lecturer with REZSŐ SOÓ in Debrecen and PÁL GREGUSS in Szeged, both of them became full professors later on. He was teaching at the KLAUZÁL Secondary School in Szeged, and worked as the deputy director of the MÓRA FERENC Museum in Szeged from 1952 until 1975. During this period he was in charge of the natural history and local history collections.

He started to collect plants in 1930, and started to investigate Szeged as a town and the Szeged countryside in 1947. His main collection areas were: Csongrád County, Trans-Tisza Region, Hajdú Region, Érmellék, the region between the Danube and the Tisza, the Visedrád Mountains, the Bakony Mountains, Sopron, Kőszeg and their surroundings, the Őrség and Baranya County. He also joined in the Tisza Research Project. He was interested in studying microscopical fungi.

He was interested in making science popular. He organized more than 50 permanent or temporary exhibitions, both scientific and for popular science. Of these the „Fehértó Exhibition" and the „Tisza Exhibition" were outstanding. He took part in making the films „Tiszavirág" and „Vadvízország".

In addition, he was also interested in zoology, and in local history and literature, as well.

He carried on his research even after retiring, and planned to write a book entitled „Countryside and plant geography of the city of Szeged and its surroundings". In it he wanted to include the names and medicinal effects of plants. However, his death in 1997 did not allow him to finish it.

He received the university reader honoris causa title in 1987. In 1975 he received the silver medal of the Labour Order and in 1996 he received the „JÁVORKA SÁNDOR Prize" from the Hungarian Biological Society.

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AN OBITUARY TO LÁSZLÓ SZALAY (1920–1997)

Dr. LÁSZLÓ SZALAY, professor emeritus of the József Attila University, founder and former head of the Department of Biophysics passed away in Szeged, Hungary on March 19, 1997. He was a distinguished scientist whose memory will be cherished by a large number of people of the university and the scientific community.

Professor SZALAY was born on December 19, 1920 in Budapest. He spent his childhood at Kakucs, village in the Great Hungarian Plain near the capital. After finishing his studies with excellent results in the secondary school of Kunszentmiklós, he was enrolled to the techers' training program in mathematics and physics at the Faculty of Natural Sciences of the University of Szeged in 1940. His interest and talent for theoretical and experimental physics was soon recognized and he became a research fellow at the Department of Experimental Physics as a third-year university student in 1943. After graduation Dr. PÁL FRÖHLICH, professor of physics at the University of Szeged appointed him to his chair as an assistant and started his high-rising scientific career. In 1947, LÁSZLÓ SZALAY passed his examination for doctorate of philosophy (Ph.D.) in physics with „summa cum laude" on the subject of molecular luminescence. He was awarded the degree of Candidate of Physical Science in 1951 and defended his academic doctoral thesis on polarized luminescence of molecules in 1964. He founded the Department of Biophysics at the József Attila University, Szeged in 1969, gave the chair a characteristic educational and scientific profile and brought it in many respects to international level. In addition to his duties at the University, he founded the Department of Biophysics of the Biological Research Center of the Hungarian Academy of Sciences in Szeged in 1971. The broad research field in photo-, neuro- and membrane biophysics, started here and partly conducted by him, became internationally recognized. From 1973, he has devoted all his time and energy to the education and research at the University.

Early in his undergraduate years, his research interest became engaged in the absorption and luminescence spectroscopy of organic dyes and he remained faithful to it all over his scientific career. Although the Department of Experimental Physics of University Szeged had some history in that field before his activity, the

World War II caused serious losses both in teaching staff and properties. First as a young assistant, later as one of the prominent leaders of the department, he contributed substantionally both to the reconstruction work and the establishment of new directions and methods in molecular spectroscopy. Dr. SZALAY was especially gifted in formulating and solving problems but was well trained in experimental works as well. He successfully joined to the work on the determination of the true luminescence characteristics of the dyes. This was not just a theoretical problem but had important practical consequences. The directly measurable fluorescence parameters (spectra, degree of polarization, quantum yield and lifetime) are sometimes heavily distored by reabsorption and secondary (tertiary etc.) emission of light. He, together with his colleagues among others professors (Á. BUDÓ and I. KETSKEMÉTY) managed to develop a method to derive the true (molecular) fluorescence characteristics from the observed macroscopic quantities, and determined the conditions where the corrections were the negligible and the measurable values delivered the true values directly. The migration of electronic excitation energy among organic dye molecules was one of his favorite topics. The basic principles of the energy transfer by inductive resonance were formulated by Th. FÖRSTER (Stuttgart) at the end of the forties. Dr. Szalay immediately recognized the importance of the Förster's theory and applied it to his polarization studies. He saw clearly how the transfer of electronic excitation energy decreased the observed degree of polarization of fluorescence. Based on his essential and principal depolarization experiments, Dr. SZALAY has become an internationally acknowledged expert on polarization and energy transfer of molecular luminescence. In the second half of the sixties, his attention turned to photobiological problems and he soon established an active workshop and later a school of photophysics/biology. He focussed his research interest to the spectroscopy of fluorescent amino acids (tyrosine, tryptophane etc.) and photosynthetic pigments (chlorophylls, carotenes etc.). He studied the role of proper spatial and spectroscopic arrangement of the light-harvesting (absorbing) pigments in the funnelling of the excitation energy to the reaction sites (centers) of photosynthetic organisms.

Although Dr. SZALAY has consistently shown up significant scientific results and has been building up connections with scientists abroad, the (political) atmosphere in Hungary and at the University did not favour the long-term research visiting status in laboratories abroad. He was already 45 years old and an acknowledged professor of biophysics when, for the first time, he could accept a one-year fellowship to the famous photosynthesis laboratory at the University of Illinois at Urbana, USA, headed by Dr. E. RABINOWITCH.

He has built up excellent cooperation with many laboratories in different countries and is highly interested in promoting the international relations among scientists. He facilitated the participation in common scientific research programs

with among others the Institute of Plant Physiology of the University of Göttingen (Dr. W. WIESSNER), the Institute of Biophysics of the University of Illinois at Urbana (Dr. C. A. WRAIGHT), the Laboratory of Photosynthesis CNRS, Gif-sur-Yvette (Dr. J. LAVOREL), the Institute of Biopysics of the Lomonosov State University of Moscow (Dr. A. RUBIN), Laboratory of Biochemistry at Moscow (Dr. A. A. KRASNOVSKY), Christie Hospital and Holt Radiation Institute at Manchester (Dr. R. DALE) and Center of Fluorescence Spectroscopy at the University of Maryland at Baltimore (Dr. J. LAKOWICZ). Dr. SZALAY was invited lecturer in a number of international congresses, guest professor in the USA (Albany, New York, 1970), in Germany (Tübingen, 1974) and in Egypt (Cairo, 1976). He was the organizer of several congresses and international symposia. Dr. SZALAY was present at the organization of the European Society for Photobiology (ESP) and worked for three years in the executive committee. In 1989, he was awarded by the precious prize of the ESP for his pioneering activity in photobiology.

Beside his research activites, he always stressed the significance of teaching. Dr. SZALAY coauthored two books (Luminescence in Biology and Medicine, 1983 and Biophysics, 1985) and edited numerous university textbooks and practice-manuals for students in medicine and biology. About ten of his co-workers have been awarded the degree of Candidate of Biological Science and at least three times more students have made the Ph. D. work under his supervision. Since his enrollment as freshman half a century ago, Dr. Szalay has attached firmly to our University. He has never left it (he kept the chair when he was the director of the Institute of Biophysics of the Biological Research Center of Szeged) although the temptation for better research conditions was sometimes very high. He served the institution on different levels: Vice-Dean (1956–59) and Dean (1969–72) of the Faculty of Natural Sciences, Pro-Rector (1965–1968) of the University and Head of the School of Life Sciences during two terms.

We will remember him as one of the happiest persons that it was our good fortune to encounter: a person whose joy of life spilled over easily into his scientific life: a person who particularly delighted in sharing his success with his family: a person whose philosophy of life was to work hard. Even in critical situations, he could find the right way to treat people. This is a rare and highly positive feature and its cohesive effect on the structure and function of the School of Life Sciences was utilized several times. He never thrusted himself forward but always did his best to help the scientific career of others. One of his striking achievements has been the realization of international cooperation with numerous laboratories, and he offered many opportunities for his co-workers. He always remained a soft-spoken man of no pretension and guided us imperceptibly to the world of science, education, culture and humanity. We will remember him as a charming host, successful organizer and participant of numerous international meetings. We can never repay him for his unstinting generosity and for his

indefatigable efforts to insure for us a relaxed atmosphere. His death leaves a void that will be difficult to fill. We lost an excellent mentor and friend.

Professor SZALAY is survived by his wife, Elizabeth and by his daughter, Judith. Our heartfelt sympathy goes out to them at the loss of their much loved husband and father.

Dr. Péter Maróti
Professor of Biophysics
Head of the Department of Biophysics
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IN MEMORIAM PROF. DR. HABIL. BÉLA MATKOVICS (1927–1998)

Dr. BÉLA MATKOVICS, retired head of the Biological Isotope Laboratory, has died at the age of 72. His death came dramatically and unexpectedly.

Béla Matkovics was born in Csongrád on 12 July 1927. He finished his elementary and secondary school studies in Bonyhád and Baja. His medical university studies were performed in Szeged between 1945 and 1951. He was qualified as a physician in 1951. In the same year he began to study chemistry, and obtained his diploma in chemistry in 1955. Between 1950 and 1955 he worked at the Department of Medical Chemistry of the University of Medical School, Szeged. His research work was focused on the microbial transformation and on the effects of steroids. His investigations were then directed towards the study of redox properties. Since 1955 he have been working at the József Attila University in Szeged. In 1956 he specialized in medical clinical chemistry. Up to 1969 he was employed at the Department of Organic Chemistry. In September 1964 he became the candidate of chemical sciences and in December 1964 he defended his university doctoral thesis.

Between 1951–1955 he was an assistant at the Department of Organic Chemistry. During this period biochemistry became a new discipline separating from organic chemistry. Dr. BÉLA MATKOVICS was the first who has started to organise the education of biochemistry at our University. Between 1955–1965 he worked as a senior lecturer and from 1965 as an associate professor in chemistry. Four years later he joined to the Biochemical and Genetical Group organized within the frame of the Biological Chairs. During this time his research interest turned into the direction of enzymes involved in oxidative metabolism. He realised in time, that oxidative processes, free radicals, mostly oxygen radicals will come into focus in the biological research. He was one of the first researchers in Hungary who has started to study the lipid peroxidation processes and the members of the antioxidant systems in living organisms. Due to a reorganisation within the Biological Chairs, the Biological Isotope Laboratory was established in 1974. Dr. BÉLA MATKOVICS bacame the head of this Laboratory where he continued the examination of the oxidative stress and the antioxidant systems.

He visited most of the European countries, gave lectures or performed research work at different Universities of Europe. In 1959 he was awarded by a fellowship of the International Atomic Energy Agency (Vienna) and spent one year at the First Department of Chemistry of the Karolinska University in Sweden, in collaboration with Prof. S. BERGSTROM and Prof. B. SAMUELSSON.

Dr. MATKOVICS maintained good relations with our Twin Universities for a long time. I mention only the 20 years old research-friendship with the Institute of Chemistry, University of Novi Sad, Yugoslavia and with the Department of Biophysics, University of Lodz, Poland. In appreciation of his work he got an award in 1985 for „Coordination of the friendship between Polish and Hungarian Universities". For education he got the „Excellent lecturer and educator" award from the Ministry of Education two times, in 1983 and 1992.

It was a great pleasure for Prof. MATKOVICS to work in different scientific societies. He was a member of the Hungarian Chemical Society from 1951, the Hungarian Biochemical Society from 1960, the Society of Free Radical Research (SFRR) from 1984. His work was honoured with „Than Karoly memorial medal" by the Hungarian Chemical Society. This year he got the „Dr. H. Falk Price" from the Society of the Free Radical Research (Europe) Hungarian Branch.

Up to now he was working as associate president of SFRR Hungarian Branch from 1991 and associate president of Hungarian Chemical Society Local Section in Szeged, from 1989.

It would be hard enough to list the name of his coworkers. There are a lot of researchers in Hungary and some in the world, who have started to build their career with generous help of Prof. MATKOVICS.

Dear Reader, you can make ensure of looking at the authors of 288 scientific articles and 38 chapters or books which were made together coworkers. Dr. BÉLA MATKOVICS presented gladly the results of his Laboratory at conferences. He hold 194 lectures.

Although Prof. MATKOVICS was retired officially from July, 1997, his everyday program did not change. He started the day in the library, then spent the day in the Laboratory. He was sorry to hear about the structural reorganisation of the Laboratory.

We got the bad news about his illness in August . . . and he left us in October.

Professor MATKOVICS provided us with countless demonstration of kindness, enthusiasm, joy, scientific rigor and pleasure in doing good science. He will stay in our memory forever.

Ilona Sz. Varga
Biological Isotope Laboratory
József Attila University, Szeged

IN MEMORIAM DR. SÁNDOR OLÁH (1960–1998)

Birth, marriage and death: these are the greatest events in human life.

Birth, marriage and death: we only have choice in the second of these, but we have no choice in birth and death.

Birth is a time of joy, death is a time of sorrow and mourning.

Events what we always remember. Events of what happiness or sorrow we want to share.

Now we want to share in our deep sorrow.

It was a great shock to hear the sad news of Sanyi's death and we are deeply grieved about it.

The Department of Anthropology of the József Attila University and the Hungarian anthropology are suffering from a heavy loss. We lost a young and well promising colleague. Dr. SÁNDOR OLÁH, retired assistant professor, died at his age of 38th.

He was born in Miskolc on 21 May, 1960. He took his primary school in Eger, than in 1978 he matured in the Dobó István Grammar School in the same town. It is possible that the spirit and historical past of this town determined his choice of profession. He continued his studies in 1979 at the Kossuth Lajos University in Debrecen where he got his degree in biology in 1984. Between 1984 and 1987 he worked for the Institute of Anatomy of the Medical University of Debrecen as an assistant researcher leading anatomical and histological laboratory practicals. He became our colleague at the Department of Anthropology of József Attila University on 1 August, 1987. He started as an assistant researcher, than between 1991 and 1992 he worked as an assistant, and in 1992 he was appointed to an assistant professor.

In 1991 he „summa cum laude" defended his doctoral thesis written on historic anthropology titled „Historic anthropological evaluation of the Conquest period (10th century) cemetery from Sárrétudvari-Hízóföld".

After having a heavy stroke on 3 September in 1994 he could never regain his health in spite of the repeated operations and because of this he was retired. He died on 9 November, 1998.

During his tragically short life while he worked 10 years as an instructor and researcher of the higher education, he proved his exceptional talent and working capacity. He devoted his interest to historic anthropology what he could intensively and successively study by the combination of his profound anatomical knowledge and modern computer skills. In addition to this he immediately gained the affection and honour of many of us by his kind character, refined behaviour, readiness to help and versatile interest.

He had fine prospects, and the merciless illness which suddenly broke his life — especially the fact that he was not able to surmount it — has staggered and shocked all who knew Him. By his forced retirement and especially by his death it became obvious that the Department of Anthropology and the Hungarian anthropology lost a brilliant, talented teacher, researcher and promising colleague. This is proved by his publications, papers presented on national and international congresses and meetings, for which he always prepared himself with exceptional soundness. His audience could always expect an enjoyable lecture what He never read.

It is hard to believe and accept the unchangeable fact, that Sanyi will never be with us, that we cannot count on some discussion with Him, that we cannot ask Him for help, that we have to miss his particular character, his friendship and everything He gave us during the time He shared us.

Dear Sándor, dear Friend of us we will never forget You, we will never bury You in our heart. Peace to your memory!

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Gy. L. Farkas

CHRONICLE IN 1997

Personalia

Ass. Prof. dr. LÁSZLÓ GALLÉ (Department of Ecology), Ass. Prof. dr. ENDRE LEHOCZKI (Department of Botany) and Ass. Prof. dr. MAGDOLNA SZENTE (Department of Comparative Physiology) have been appointed Professors by the President of the Hungarian Republic.

Vice-manager dr. ÁRPÁD PÁRDUCZ (Biological Research Center of Hungarian Academy of Sciences, Szeged) have been appointed Privat docent by the President of the Hungarian Republic.

Prof. dr. JÓZSEF TOLDI (Department of Compararative Physiology), Ass. Prof. dr. FERENC KEVEI (Department of Microbiology) and Ass. Prof. dr. ANTÓNIA MARCSIK (Department of Anthropology) have been appointed to the chairs of those Departments by the Rector of J. A. University.

Ass. Prof. dr. ERZSÉBET MIHALIK have been appointed to the president of Biological Section of the Faculty of Natural Sciences of J. A. University by the Rector of J. A. University.

Awards

Prof. dr. LAJOS FERENCZY, Member of the Hungarian Academy (Department of Microbiology) and Prof. dr. OTTÓ FEHÉR (Department of Comparative Physiology have been awarded the „József Attila Medallion" by the József Attila University.

Habilitation proceedings

In 1997 the following application lecture for habilitation were presented at the Biological Section of the Faculty of Natural Sciences:

Ass. Prof. dr. LÁSZLÓ GALLÉ (Department of Ecology): Competition between of Populations.

Conference

Prof. Dr. FERENC ZSOLDOS, Professor Emeritus of József Attila University Szeged, is 70 years old. On the occasion of his birthday, colleagues, and the leader professors of the biological departments congratulated FERENC ZSOLDOS, the former Chairman of the Department of Plant Physiology in 4th April 1997. In the frame of this celebration Prof. Dr. LÁSZLÓ ERDEI, present Chairman of the Department of Plant Physiology, summarised his course of life in a cordial speech. Paying him respect, the youngest colleagues, starting their career, presented lectures (KRISZTINA FÜLÖP: *The antioxidant protection system of plants and environmental stress;* FERENC HORVÁTH: *Set-up of the new „patch clamp" laboratory*).

CHRONICLE IN 1998

Personalia

Dr. LÁSZLÓ KÖRMÖCZI (Department of Ecology) have been Ass. Prof. by the Rector of J. A. University.

Awards

Prof. dr. OLIVIER DUTOUR (Laboratoire d'Anthropologie-Biologique — CNRS UMR 6578, Université de la Méditerranée, Marseille, France) and Prof. dr. OTTÓ G. EIBEN (Department of Anthropology, Eötvös Lóránd Univerrsity, Budapest) have been awarded the „Bartucz Lajos Medallion" by the of József Attila University.

ILONA RICHTER Munkácsy Prize winner grafic artist have been awarded the „József Attila Medaillon" by the József Attila University.

Prof. Dr. GYULA L. FARKAS, President of the Hungarian Biological Society of Szeged (Department of Anthropology) have been awarded with the „Gelei József prize" by the Presidium of Hungarian Biological Society.

Habilitation proceeding

In 1998 the following application lecture for habilitation were presented at the Biological Section of the Faculty of Natural Sciences:

Ass. Prof. dr. KORNÉL KOVÁCS (Department of Biotechnology): Characterisation of the stable /NiFe) hyrogenase and the properties influencing structural stabilities.

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